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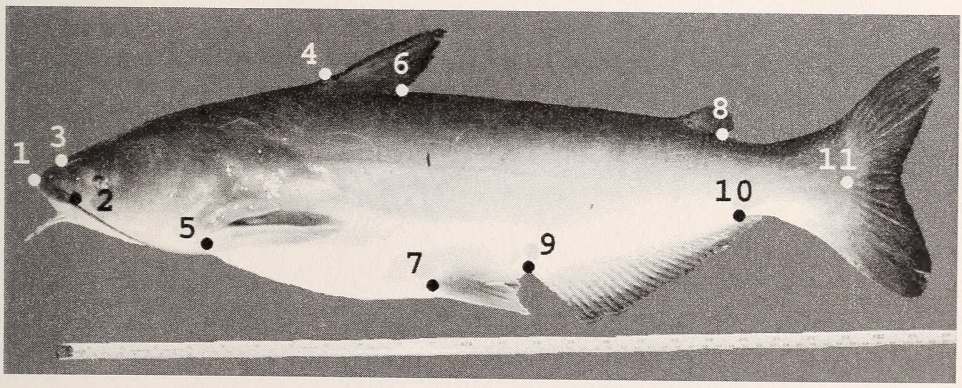

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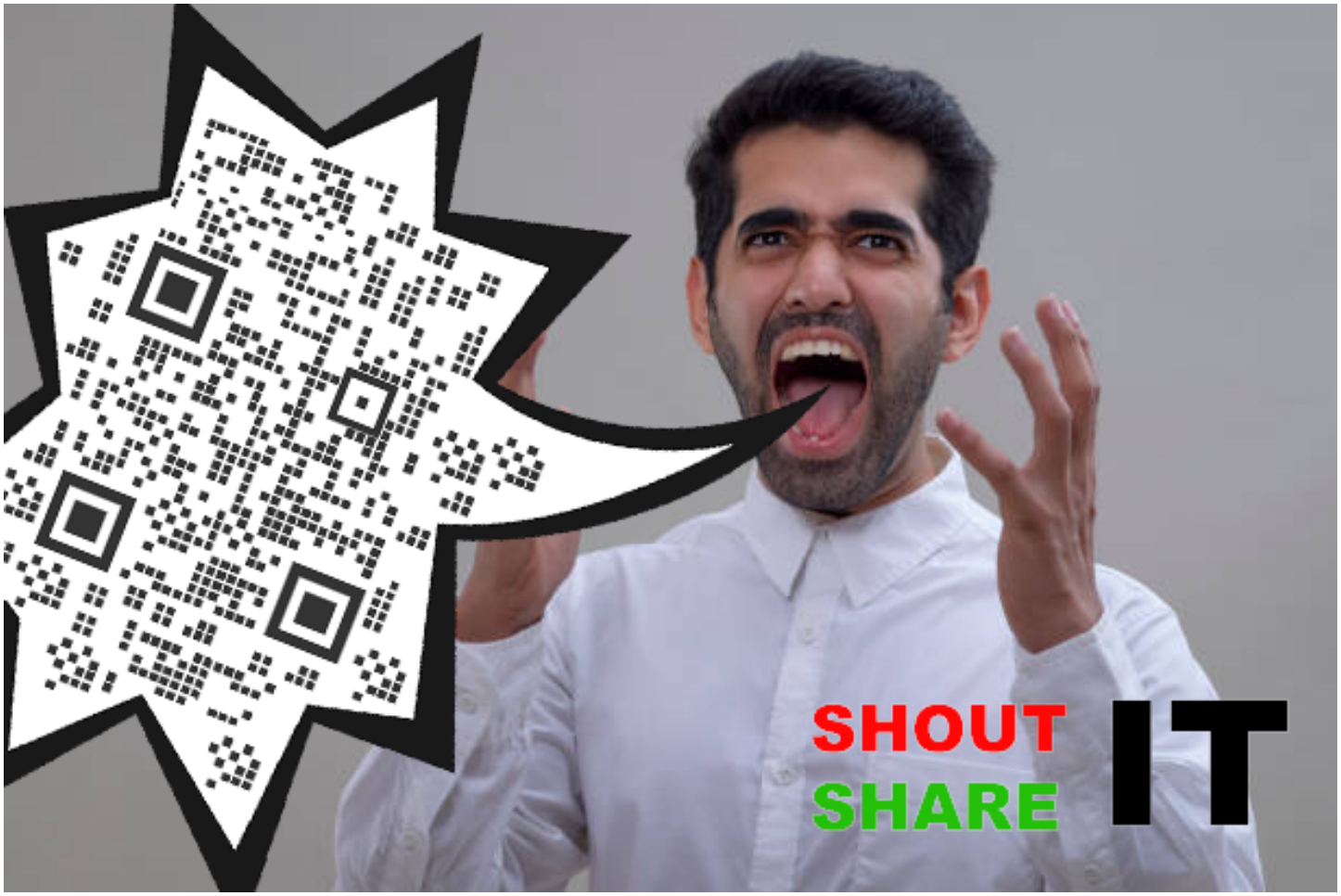
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Date of this issue 29 April 2009

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Annual Meeting of the Southern California Academy of Sciences

Marymount College, May 29–30, 2009

FIRST CALL FOR SYMPOSIA AND PAPERS

The Southern California Academy of Sciences will hold its annual Meeting for 2009 on the campus of Marymount College, Palos Verdes Friday and Saturday May 29–30.

Presently the following symposia are in the planning stages. If you would like to organize a Symposia for this meeting, or have suggestions for a symposia topic, please contact An Dalkey adalkey@pvplc.org or John Roberts at jroberts@csudh.edu. Organizers should have a list of participants and a plan for reaching the targeted audience.

Note: Abstracts will be due on April 17, 2009. Check our web page for further information (http://scas.jsd.claremont.edu/)

FRIDAY, MAY 29: Planned Symposia

Sustainable Fisheries: organized by Mark Helvey (Mark.Helvey@noaa.gov)

Soft Bottom Marine Ecology: organized by M. James Allen (jima@sscwrp.org)

Sustainability and Climate Change: organized by Ann Dalkey (adalkey@pvplc.org)

Poster Session: 5 p.m.

SATURDAY, May 30: Planned Symposia

Reef Biology: organized by Bob Grove (grovers@sce.com) and Dan Pondella (pondella@oxy.edu)

Marine Platforms: organized by Chris Lowe (clowe@csulb.edu)

Southern California Paleontology: organized by Mark Roeder (maroeder 1731@aol.com) and Sarah Siren

Southern California Archaeology: organized by Andrea Murray (apmurray@pasadena.edu)

Contributed papers: Sessions of Contributed Papers will occur both days.

Plenary Sessions: Friday, Bill Patzert (Jet Propulsion Labs.)

"Oceans Under Stress... The Future Ain't What It Used To Be"

Saturday, Ann Bull, Minerals Management Service "Outlook on the Outer Continental shelf for Offshore Drilling"

Contributed Papers and Posters: Both professionals and students are welcome to submit abstracts for a paper or poster in any area of science. Abstracts are required for all papers, as well as posters, and must be submitted in the format listed on the society webpage. Maximum poster size is 32 × 40 inches.

In addition **Junior Academy members** will submit papers for Saturday sessions.

Abstracts of presented papers and posters will be published as a supplement to the August 2009 issue of the Bulletin.

Student Awards: Students who elect to participate are eligible for best paper or poster awards in the following categories: ecology and evolution, molecular biology,genetics and physiology, and physical sciences. In addition the American Institute of Fishery Research Biologists will award best paper and poster in fisheries biology. A paper by any combination of student and professional co-authors will be considered eligible provided that it represents work done principally by student(s). In the case of an award to a co-authored paper, the monetary award and a one year student membership to the Academy will be made to the first author only.

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Die Off and Current Status of Southern Steelhead Trout (*Oncorhynchus mykiss*) in Malibu Creek, Los Angeles County, USA

Rosi Dagit,[1] Stevie Adams,[2] and Sabrina Drill[3]

[1,2]*Resource Conservation District of the Santa Monica Mountains, P.O. Box 638, Agoura Hills, CA 91376-0638*
[3]*University of California Cooperative Extension, Los Angeles and Ventura Counties, 4800 Cesar Chavez Blvd., Los Angeles, CA 90022*

Abstract.—A die-off of native and exotic fish and invertebrate species, including the endangered southern steelhead trout (*Oncorhynchus mykiss*) was observed in Malibu Creek, Los Angeles County, during the summer and fall of 2006. Death was preceded by a period of illness during which trout in particular exhibited a noticeable yellow coloration. Physical, chemical and biological variables, including temperature, dissolved oxygen, a variety of chemical contaminants, presence of toxin producing algae, and direct pathology were examined but results remain inconclusive. The first day of a 12-day high temperature event occurred on the same date yellow trout were first observed. This sustained event is different from shorter term temperature spikes recorded in other years. Recovery monitoring documented re-colonization by all exotic fish species and crayfish, but limited numbers of southern steelhead trout in 2007. Surveys in summer 2008 documented a record number of anadromous adults (five silvery fish over 50 cm total length) and young of the year (over 2,200 under 10 cm).

Malibu Creek in Los Angeles County is home to one of the southernmost reproducing populations of the endangered southern steelhead trout (*Oncorhynchus mykiss*). Located in arid southern California, this stream offers access to the ocean only during the rainy winter season when high flows breach a sand berm across the mouth of the estuary. Adults entering the stream from the ocean reach an impassible barrier presented by the 30 meter high Rindge Dam, restricting them to the lowest 3.2 km of more than 112 km of historic steelhead habitat. The dam, originally built for water storage and flood control in 1926, no longer functions as the reservoir is completely filled with sediment. Over the past decade several government agencies and non-profit organizations have been trying to remove the dam to restore access to upstream spawning habitat. In 1997, the southern Evolutionarily Significant Unit (ESU) of steelhead trout was added to the federal list of endangered species, with Malibu Creek as the southernmost boundary. Since 1997, the protected range of this ESU has been extended to the U.S./Mexican border. The National Marine Fisheries Service (NMFS) estimates that only 500 anadromous adults remain within this ESU (NMFS 2007).

Located within the 283sq kilometer Malibu Creek watershed (Figure 1), Malibu Creek and its tributaries are impacted by source and non-point source pollutants emerging from the residential, commercial, animal husbandry and infrastructure facilities covering at least 22% of the total area (Dagit et al 2005). Malibu Creek has been placed on the federal list of impaired water bodies under section 303 of the Clean Water Act due to water

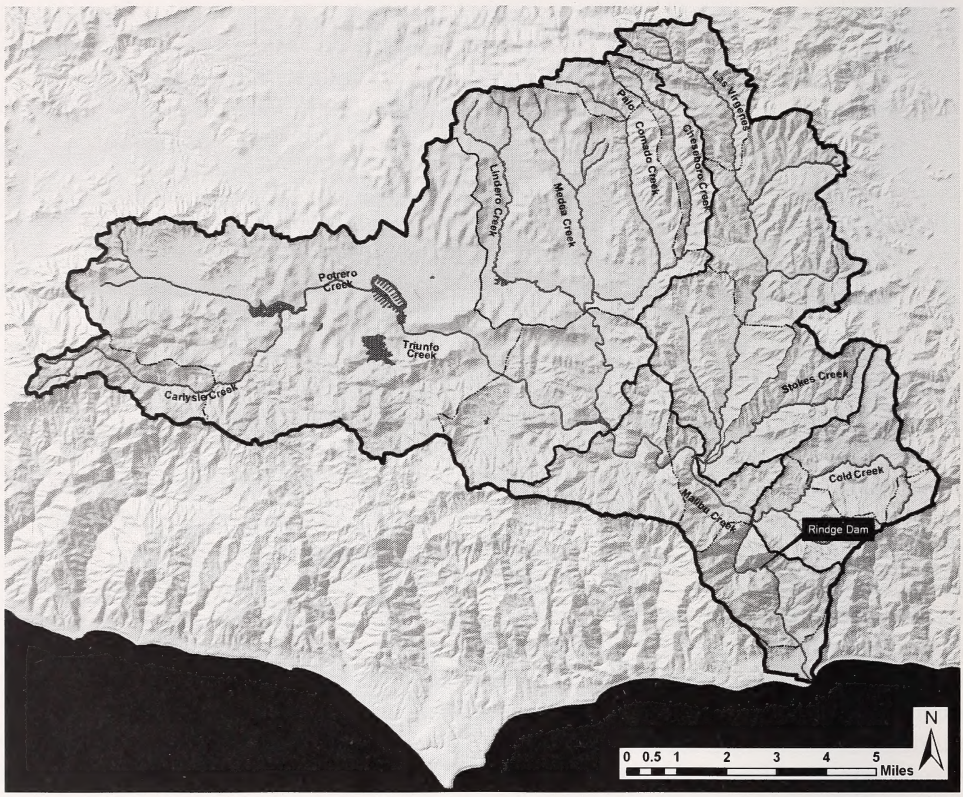

Fig. 1. Malibu Creek Watershed map, Study reach is located downstream of Rindge Dam (Map provided by M. Grimmer, Heal the Bay).

quality degradation related to excess sedimentation, nutrients, coliform bacteria and pathogens, trash, scum and foam (LARWQCB 2007). The creek is also host to numerous invasive exotic species, including red swamp crayfish (*Procambarus clarkii*), common carp (*Cyprinus carpio*), and recently introduced New Zealand mud snails (*Potamopyrgus antipodarum*) (Dagit and Abramson 2007). In recent years we have noticed a sulfurous unpleasant odor in portions of the creek, and the substrate in shallow pools is often covered with a slimy black muck that may itself be covered with a white/grey powdery looking substance.

The Resource Conservation District (RCD) of the Santa Monica Mountains has conducted monthly snorkel surveys to monitor the *O. mykiss* population in Malibu Creek since June 2005. In May 2006, we recorded the highest numbers of *O. mykiss* in Malibu Creek seen since the surveys began (245 individuals including more than 70 young-of-the year). In July 2006 we observed a yellow coloration in a few otherwise healthy small (< 15 cm) juvenile trout. The next month, yellow trout of all sizes were observed, and appeared stressed or sick, swimming sluggishly with mouths agape. In September, only nine *O. mykiss* were observed in Malibu Creek. Other species including crayfish, carp, and catfish, while not exhibiting the yellow coloration, had declined in number and showed similar sluggishness. By November 2006, no fishes at all were found in Malibu Creek.

In December 2006, a Technical Advisory Committee was convened to frame an investigation into the possible causes of the yellow coloration observed in *O. mykiss*, and

the morbidity and mortality observed among these fish and other aquatic species. This group included aquatic biologists, chemists, and other experts from the RCD of the Santa Monica Mountains, University of California Cooperative Extension, California Department of Parks and Recreation, the National Park Service – Santa Monica Mountains National Recreation Area, California Department of Fish and Game (CDFG), NMFS, Las Virgenes Municipal Water District (LVMWD), Malibu Creek Watershed Monitoring Program/City of Calabasas, UC Santa Barbara, UC Los Angeles, California State University-San Marcos, Heal the Bay, Los Angeles County Vector Control (LACVC), and the Los Angeles Regional Water Quality Control Board (LARWQCB).

This group collected and reviewed existing data and developed the following list of questions for further study:

1. What caused the yellow coloration?
2. What caused the morbidity and mortality of the trout and other aquatic species?
3. Was there an unusual water quality problem such as low dissolved oxygen, extreme nutrient loading, or extreme high temperatures?
4. Did the recent invasion by New Zealand mud snails (*P. antipodarum)* play a role?
5. Did the thick layer of muck covering the channel substrate contribute to the morbidity and mortality, and what was the composition of this material?

These questions guided investigation into the die-off. All of these questions were not conclusively answered, but several variables were identified that should be helpful in developing a quick response research program if this phenomenon is observed again.

Materials and Methods

Snorkel surveys were conducted monthly from June 2005 to June 2007 between the upper extent of Malibu Lagoon (upstream of Cross Creek Road) and Rindge Dam, covering approximately 3.2 km of stream reach. An additional reach (500 m upstream of the dam) was added to the survey from February–June 2007 so that we could observe the status of exotic fish species density where no die-off was documented. Additional monthly surveys were done between June and October 2008.

Surveys were conducted by a trained team of 3–4 divers and one observer/data recorder. Divers enter the water on the downstream end of the area and slowly work their way upstream searching under every boulder undercut, bubble curtain or other instream feature where fish might hide. The observer is positioned with a clear view into the water in order to see fish, and determine if any fish swim away from the divers. Each habitat unit where fish are located is characterized by type (pool, run, riffle), depth, width, dominant substrate, canopy cover, instream cover, and shelter value, according to the standards of the *California Salmonid Stream Habitat Restoration Manual* (Flosi and Reynolds 1998). Large, deep pools were often surveyed twice by divers swimming in a transect formation, first from the surface, and then again after about 30 minutes, with divers inspecting every undercut as well. Counts were verified by the observer, who discussed the number and condition of fish observed with each diver to avoid recording duplicate fish observations from different divers.

Water temperature was measured every 15–30 minutes by use of in-stream recording thermometers (Stowaway Tidbits, HOBO, Inc.). Loggers were placed in two locations each year where trout were consistently observed (Malibu dam pool-2005, 2006, 2008, lower twin pool-2008, and just downstream of the start pool- 2005, 2006) within the study reach (Figure 2). These two locations were selected since each represented conditions in

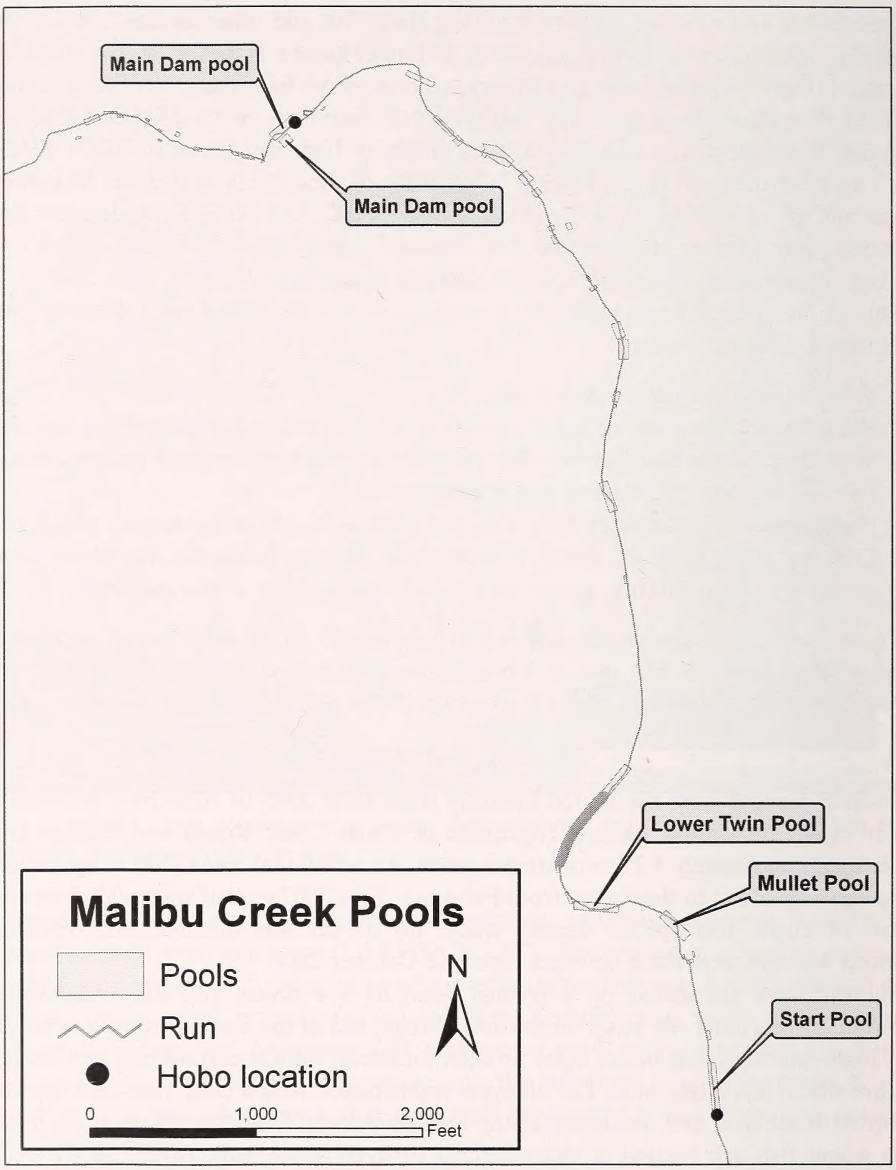

Fig. 2. Snorkel Survey reach showing pool locations, Malibu Creek. The start pool is approximately 500 m upstream of the upper end of Malibu Lagoon. (Map provided by M. Grimmer, Heal the Bay).

the upper and lower reach of the study area. The dam pool has continuous flow under even extreme drought conditions when portions of the lower reach go dry or are shallow and experience greater temperature fluxes.

Data were collected in the start pool from July 29–October 21, 2005. Breaks in the data occurred when the logger was exposed to air. The logger in the dam pool was stolen prior to downloading in 2005. In 2006, a logger in the dam pool collected data from May 18–July 19 (it was then stolen), and in the start pool from July 18–September 12. In 2008, the logger was installed in lower twin pool (start pool was too shallow) from 13 June–July 24 (when the logger was stolen) and in the dam pool from June 13–September 10.

All temperature data were examined and outliers were removed if we had evidence (based on either field observations or data irregularities) that the logger had been recording while out of the water. Daily maximum and mean temperatures were calculated. Data were also examined to determine proportion of time that water temperatures exceeded 27.5 degrees C at each location.

Grab samples were collected monthly in 2005–2006 by the Heal The Bay Malibu Stream Team just below the start pool (HtB- 1) and above the influence of the Tapia Wastewater Treatment Plant located approximately 1.6 km upstream of Rindge Dam, and tested for nitrate-N, ammonia- N, orthophosphate, pH, turbidity, and conductivity. The LVMWD also collects water quality data regularly from the outfall of the Tapia Wastewater Treatment Plant. In September 2006, samples were specifically collected and tested for a suite of toxic pollutants and heavy metals. A chronic bioassay test on *Ceriodaphnia* and Fathead minnow with copper chloride control was also conducted (Aquatic Bioassay Report 2006).

Application records for the mosquito larvicide *Bacillus thuringienses isrealensis* (Bti) were obtained from the LACVC for the four locations within or affecting the study reach where the fish die-off occurred.

In December 2006, sediment samples were collected and tested for toxicity using the freshwater amphipod *Hyalella azteca*, by the Nautilus Environmental Bioassay Laboratory, San Diego, CA.

Grab samples of the muck were collected in three locations (start pool, mullet pool and lunch pool) in August, September and December 2006. Each sample included both the surface scum of white, and the more dominant black, sulfurous smelling bulk. The muck felt gelatinous, and dispersed into a cloud when touched. Samples were kept cool and transported for examination by microscope by Dr. Robert Sheath at CSU San Marcos, Robert Gilbert at UCLA, and Dr. Tom Dudley at UC Santa Barbara.

In January 2007, over 100 New Zealand mud snails and 10 native snails were collected alive from the impacted study reach. Additional live samples were collected upstream in Malibu Creek State Park, where no fish die-off was observed. These snails were examined for trematode infestations by Dr. Brian Fredensborg at UC Santa Barbara.

A total of three *O. mykiss* were caught by hook and line in September 2006 under the supervision of the National Oceanographic and Atmospheric Administration (NOAA) Office of Law Enforcement. Gross pathology, parasitology, bacteriology and histology tests were performed by CDFG Fish Health Laboratory veterinarian Dr. Joe Maret, both in the field and in subsequent laboratory efforts. Dr. Ron Hedrick (UC Davis) reviewed and commented on the histology.

Results

Snorkel Surveys

In May 2006, a total of 245 trout were observed, along with the usual suite of exotic species dominated by crayfish, carp, bluegill (*Lepomis macrochirus*), green sunfish (*L. cyanellus*), black bullhead (*Ameiurus melas*), mosquitofish (*Gambusia affinis*), fathead minnow (*Pimephales promelas*), and largemouth bass (*Micropterus salmoides*). That same month, New Zealand Mud Snails were identified in a macroinvertebrate sample from Malibu Creek that had been collected in 2005 by Heal the Bay. Researchers from a variety of agencies working in streams in the Santa Monica Mountains voluntarily suspended field work until decontamination procedures for field equipment and clothing were established to prevent spread of the snails. Hence, no snorkel survey was conducted

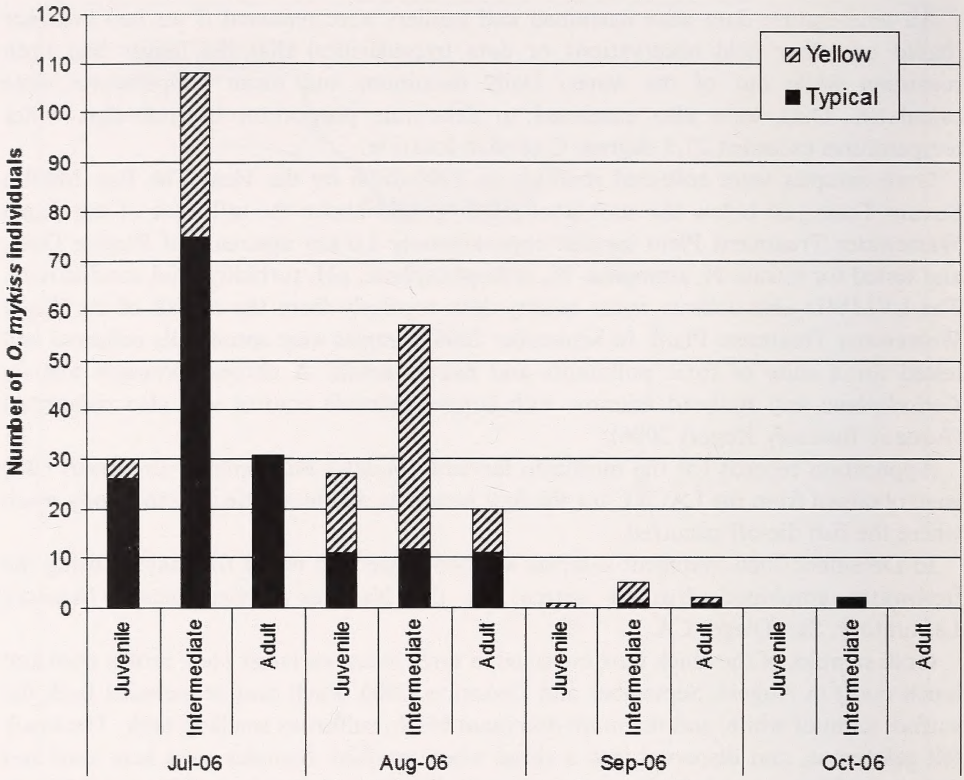

Fig. 3. Yellow coloration progressed through size classes of *O. mykiss* in July and August 2006. In September 2006, abundance decreased dramatically and by November 2006 no fish of any species were observed in the study area of Malibu Creek. Juvenile = 0–10 cm, Intermediate = 11–25 cm, Adult > 26 cm total length.

in Malibu Creek in June 2006. During the July 18–19 2006 survey, we observed 37 trout with yellow coloration, all under 15 cm, as well as another 145 trout with normal coloration in all size classes. This corresponded to the start date of a 12-day high temperature episode (described below).

In August 2006, the number of trout observed exhibiting normal coloration was 36, and the yellow trout of all size classes increased to 75. Dead and dying crayfish were noted throughout the study area and a 75 cm carp that appeared ill was caught by hand. In September 2006 few fish of any species were observed. A total of nine trout were observed, of which seven exhibited the yellow coloration. After collecting three trout for pathology and histological testing in September, only two normal trout were observed in October (Figure 3). From November 2006 until March 2007, no trout and only a few carp were seen (Figure 4). The lagoon was open when the berm was breached for much of this time, allowing both in and out migration.

In April 2007, 11 trout between 35–65 cm were observed, still retaining the steel gray color indicating recent movement from the ocean. In June 2007, we counted a total of 32 trout, including eight young of the year, indicating that some spawning had occurred. Unfortunately, the rebound of exotic fishes and crayfish populations were explosive, with crayfish literally covering the bottom in some pools. Due to funding constraints, surveys were suspended until September 2007, when a total of 30 trout were recorded. Of these 30

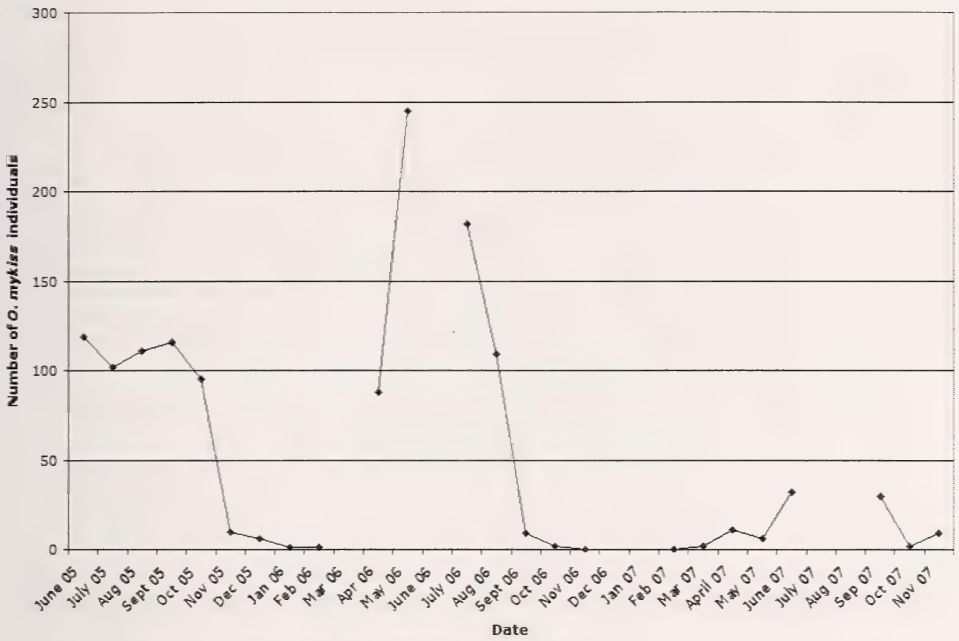

Fig. 4. Abundance of *O. mykiss* in Malibu Creek June 2005–November 2007.

trout, three adults (over 30 cm total length) were quite pale in color and one adult exhibited the same yellow color and sluggish behavior observed the previous year. By November 2007, the number of trout observed had dropped to nine adult fish, possibly due to reduced visibility during the survey. A subsequent stream walk survey done in January 2008 by CDFG biologists observed a total of five adult trout and a recently excavated redd (McKibbin, 2008).

Snorkel surveys conducted in June and July 2008 counted over 2,500 trout, with fish under 10 cm most abundant (2,293 in June and 2,327 in July). In June surveys, 24 adults over 50 cm long were observed, compared to five in July. The remainder of the fish ranged from 10–25 cm in length. Invasive non-native species have also rebounded, attaining abundances similar to those observed before the die-off event.

Flow

Flow was continuous throughout the study area and to the ocean during the entire die-off event, with levels ranging between 0.141–0.297 cubic meters/second (cms), with mean flows of 0.2175 cms in July 2006, 0.1662 cms in August and 0.1494 cms in September 2006.

Water temperature

In 2006, water temperatures ranged between 15.68–29.15 degrees C (Figure 5). Peak temperatures were observed from July 18–30, when water temperatures exceeded 27.5 degrees C, a temperature commonly considered to be near the upper end of the range of *O. mykiss*, for much of this 12-day period (Table 1). The proportion of the time in which temperatures exceeded 27.5 degrees C was much greater in 2006 than were observed in either 2005 or 2008 (Figure 6). Table 2 compares the percent time at each 2-degree temperature interval recorded in 2005, 2006, 2008. No temperature data were collected in 2007. The 2006 data set consists of information generated from two different reaches of

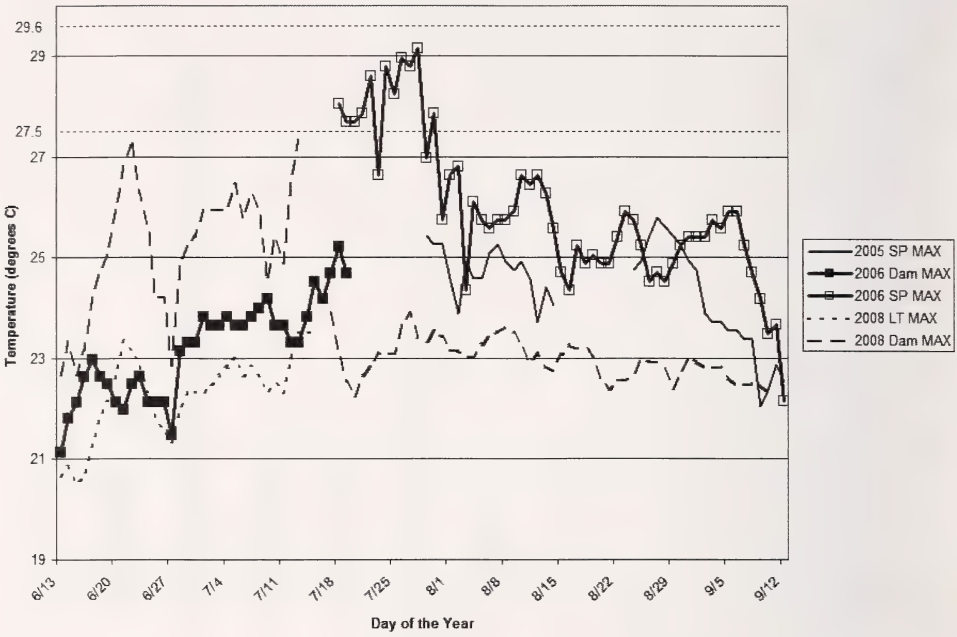

Fig. 5. Daily maximum and mean of Water Temperatures recorded in Malibu Creek from June–September 2005, 2006, 2008.

the creek because the logger in the dam was stolen following the July 19 download. It is not clear if the lower maximum temperatures observed in the dam pool prior to July 18 also reflect conditions downstream at the start pool.

Water Quality

Nutrient levels documented by HTB during the months of the fish die off did not differ significantly from levels regularly documented in recent years. Using its site HTB-1, which is located at the downstream end of the study reach just below the start pool (Figure 2), nitrate-nitrites ranged from a high of 13.5 ppm with a mean value of 2.76 ppm

Table 1. Duration of time that logged water temperatures equaled or exceeded 27.5°C, 2006.

Date	Hours >27.5°C	Hours >28°C	Hours >29°C
7/18/06	4	1.5	0
7/19/06	2	0	0
7/20/16	2.5	0	0
7/21/06	3	0	0
7/22/06	6	4	0
7/23/06	0	0	0
7/24/06	7	5.5	0
7/25/06	7	3	0
7/26/06	10	6.5	0
7/27/06	8	6	0
7/28/06	10	8	1.5
7/29/06	0	0	0
7/30/06	4	0	0

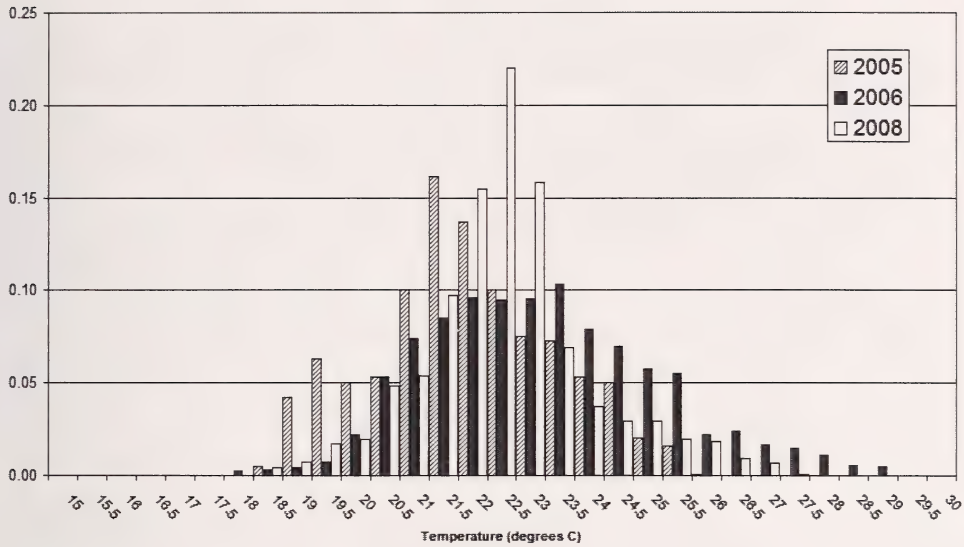

Fig. 6. Frequency of occurrence of water temperatures measured at 15–30 minute intervals in steelhead occupied pools in Malibu Creek, 2005, 2006, 2008 (2005 N=1,791; 2006 N=4,445; 2008 N=8,750).

during the fish die-off time frame. Ammonia-nitrogen levels were 0.35 ppm during August 2006, and orthophosphate levels were 0.56 ppm. A summary of all data is found at www.healthebay.org/streamteam/data/chem/query.

Dissolved oxygen levels were only directly measured mid-day at the surface, and were found to be 10 mg/l. No continually recorded DO data sets are available for Malibu Creek.

No evidence could be found for any toxic spills or acute pollution event. Toxicity testing of the grab sample collected on September 21, 2006 by the LVMWD was negative.

Bti application by the LACVC followed the same procedures as previous years, with a volume of 0.4841–0.9682 liters per event released at each of four locations within the study reach. Two of these locations received applications at the normal rate between June and August 2006 (M6 and M11)(LACVC 2006).

Sediment Toxicity

At the request of the Technical Advisory Committee, a sample of sediment was collected on 14 December 2006, refrigerated and shipped to Nautilus Environmental Bioassay Laboratory, San Diego for analysis. Mean survival of the amphipod *Hyallela*

Table 2. Percentage of logged time that water temperatures occurred in Malibu Creek between June 13–September 12 2005, 2006, 2008. (2005 N=1,791; 2006 N=4,445; 2008 N=8,750).

Degrees C	2005	2006	2008
<16.75	0%	0%	0%
16.75–18.75	1%	1%	0%
18.75–20.75	21%	9%	9%
20.75–22.75	50%	35%	53%
22.75–24.75	25%	35%	29%
24.75–26.75	4%	16%	8%
>26.75	0%	5%	1%

Table 3. Taxa found in Malibu muck samples collected in 2006.

1.	*Enteromorpha clathrata* (green algae)
2.	*Pleurosira laevis* (marine diatom)
3.	*Phormidium retzii* (cyanobacteria)
4.	*Oscillatoria tenuis* (cyanobacteria)
5.	*Scenedesmus quadricauda* (green algae)
6.	*Closterium sp.* (freshwater green algae)
7.	*Amphora sp.*(diatom)
8.	*Entonomeis sp.*
9.	*Cymbella sp.* (diatom)
10.	*Synedra sp.* (diatom)
11.	*Frustulia sp.* (diatom)
12.	*Spirogyra sp. 2* (green algae)
13.	*Cladophora cf. glomerata* (green algae)
14.	*Epithemia sp.* (diatom)
15.	*Cocconeis sp.*(diatom)

azteca in the sediment control was 92%, and mean survival of the test sediment was 98%. No toxicity was evident. (Nautilus Environmental 2007).

Muck Composition

Examination revealed a diverse community of both high and low ionic condition periphyton species, which are common to nutrient enriched environments such as that found in Malibu Creek (Table 3). No unusual or toxic species were noted (T. Dudley; R. Gilbert, R. Nhemh, unpublished data).

Pathology and Histology Results

Three adult trout ranging in size from 15–30 cm total length were captured by hook and line. All fish were examined alive and then killed for further examination. All fish appeared normal. The stomach of the 30 cm female was full of green algae. Anchor worm, a common skin parasite, was found on two of the three fish. Two of the three fish exhibited unusual inflammation of the olfactory rosette, which was not explainable. Digenetic trematode metacercariae were encysted in the dermis sections of their heads. Unfortunately, it was not possible to identify the species of metacercariae to determine if it was associated with a vector of native or non-native snails (Maret 2006). Otoliths were destroyed in the preparation of the histologic samples, so age was undetermined. Fin clips were sent to the NMFS Genetic Tissue Laboratory in Santa Cruz, CA for analysis.

Snail Examination

In order to address the question of whether the metacercariae were associated with native or non-native snail vectors, over 100 New Zealand mud snails and 10 native snails were collected from two locations in Malibu Creek in February 2007. No trematodes were found (Lund Fredensborg, pers. comm.)

Discussion

Despite concerted effort, it has not been possible to assign a single causal agent to the total loss of all species in lower Malibu Creek during the die-off period of July–December 2006. It seems unlikely that high temperatures alone explain the die-off. Infectious agents are an unlikely causative agent due to the wide taxonomic range of organisms (from

vertebrates to arthropods) affected. Based on die-offs observed in other locations, dissolved oxygen remains notable as a potential stressor, and low DO levels, combined with seasonally high water temperatures and consistently high levels of nutrients found in the water, could have led to the die-off. However, stream flow was continuous from July–December 2006, indicating that mixing was occurring and all habitat units were hydrologically connected. Of all possible contributing factors identified, the 12-day high temperature episode in July 2006 and the presence of the muck covering all wetted substrate, appear to be the two variables that are different from conditions observed in other years.

While we could find no accounts of the yellow coloration previously occurring in trout from Malibu Creek or other streams in Southern California, pale yellowish coloration has been noted in *O. mykiss* from other watersheds. Yellow trout were observed in the Salinas River during snorkel surveys in 2006–2007, and were associated with high temperature conditions (L. Thompson, pers. comm.). A study of summer water temperature conditions in the Eel River system also contained observations of both live and deceased yellow trout correlated with temperatures of between 28 and 30+ degrees C (Kubicek 1977), and this same study discussed previous sightings of yellow trout (Wales 1938, as described in Kubicek 1977). In 1938, Wales observed yellow trout in the Eel River associated with a heat wave. Wales attributed the yellow coloration to a loss of melanophore control due to disease or parasitism, but was unable to identify any causative agent.

Other observations of yellow coloration in trout have been associated with genetic mutation (Dobosz et al. 2000), though given the progression of this phenotype through the population, and the correlation with illness, we do not think this played a role in Malibu Creek.

Fish physiologists recognize that extreme heat stress could cause a loss of coloration in salmonids (Cech, pers. comm., Cech et al. 1990). Unlike the compromised water quality found in Malibu Creek, the Eel River was thought to have high water quality when yellow trout were observed. In situations where non-lethal levels of toxicants or pathogens are present along with high temperature conditions, it is possible that the cumulative impacts could result in a loss of melanophore control, stress related illness, and subsequent death even at temperatures below 28.0 degrees C.

Parasite infection such as the trematode infection observed in two of the three trout autopsied could add to the cumulative stress impacts, though it would appear that parasitism alone was not sufficient to cause death (J. Maret; J. Cech, pers. comm.). Parasitism could however be a factor contributing to a reduced ability to withstand environmental stresses, as has been observed in a related species (Keefer et al, 2007).

Thermal stress is a chronic concern in small southern California coastal streams that support these endangered fishes. In 2006, temperatures in Malibu Creek were observed to remain at the upper end of the documented thermal limits of *O. mykiss* for a 12-day period in July, with spikes of over 27.5 degrees C for up to 10 hours at a time. However, it remained below 29.6 degrees C, the critical thermal maximum observed for steelhead acclimated to high temperatures (19 degrees C) (Myrick and Cech, 2005; for a detailed discussion of the temperature, tolerances of southern steelhead see Spina 2007).

The 2006 high temperature event differs from the temperature patterns observed in 2005 and 2008 when fish mortality was not observed. In 2005, the temperature never exceeded 26.75 degrees C, and was over 22.75 degrees C for less than 30% of the time between July 29 and October 21. In 2008 short spikes of higher temperatures were

observed less than 1% of the time between July and September, concurrent with the presence of thousands of young of the year and larger trout. This contrasts with 2006, when temperatures remained over 27.5 degrees C for between 2–10 hours daily, starting on the same day that the first yellow trout were observed. Unfortunately, we do not have temperature data from 2006 from this reach prior to July 18, so it is not possible to determine if the yellow trout were responding to a previous or perhaps more prolonged high temperature event, but the data from the dam pool suggests that water temperatures in the creek were lower. It is important to note that the percentage and size class of yellow trout increased significantly in the months that followed this prolonged high temperature event, as did mortality.

O. mykiss in other southern California streams have been found to withstand similar temperatures to those we observed. Data from nearby Topanga Creek (2004–2006) indicates that resident fish routinely survive daily spikes of up to 26 degrees C (observed in late afternoon), although during the months of May through October the daily averages are closer to 18–20 degrees C (Dagit et al 2007). Carapanzano, (1996) recorded maximum temperatures up to 28 degrees C in the Ventura River. Researchers working in Sespe Creek in Ventura County also documented temperatures reaching 28 degrees C for short time periods in refugia pools where *O. mykiss* remained for most of the summer (Matthews and Berg 1997). Spina (2007) determined that juvenile steelhead were able to forage and remain active with an elevated body temperature, supporting the idea that *O. mykiss* found in the southern California region may be able to withstand, and possibly even thrive, in temperatures that would stress steelhead from cooler areas.

Despite their higher temperature tolerance, exotic species observed in Malibu Creek were found to die off along with *O. mykiss* in 2006. Temperature ranges for these species vary as described in Moyle (2002), but are consistently in line with or higher than the maxima we observed in 2006, and higher than those reported in the literature for *O. mykiss*. Common carp prefer temperatures from 4–24 degrees C, but can withstand temperatures between 31–36 degrees. Bluegill can survive temperatures as high as 40–41 degrees C but prefer temperatures between 27–32 degrees C and green sunfish prefer temperatures between 26–30 degrees C, but can survive high temperatures >38 degrees C. Black bullhead can survive temperatures up to 38 degrees C under laboratory conditions. Mosquitofish can occur at temperatures between 0.5 and 42 degrees C, but persist at temperatures between 10–35 degrees C. Fathead minnow prefer temperatures between 22–23 degrees C, but can withstand temps up to 33 degrees C. Optimal temps for largemouth bass are 25–30 degrees C, but they can handle temperatures ranging from 10–35 degrees C. Red swamp crayfish can withstand temperatures from 5–38 degrees C (Wizen, et al, 2008).

O. mykiss in Southern California and other areas are routinely observed living in warm streams, and laboratory tests confirmed their ability to acclimate to higher temperatures. However, the temperatures observed in Malibu Creek during most of the 2006 summer season were at the high end of the temperature tolerance of this species, and exceeded 27.5 degrees C for 12 days. Malibu Creek trout did not exhibit visible signs of stress, yellow coloration, or mortality in 2005 or 2008 when temperatures were slightly cooler, but still above 24 degrees C for 4% and 9% of the summer. Exotic species with significantly higher temperature tolerances also died only in 2006. Therefore, we do not think that heat stress alone can explain the die-off, but likely contributed to sensitivity to other factors.

While Southern California trout may be able to tolerate exposure to high temperatures, it still appears that they will select for lower temperatures and higher oxygen content when these conditions are available (Santa Ynez River Technical Advisory Committee

2000). Dissolved oxygen levels were not measured during the die-off, although flow was continuous, potentially indicating that oxygen levels might have been maintained through physical mixing. In Sespe Creek, pools, or sections of pools having low dissolved oxygen levels were avoided by *O. mykiss*, who selected for pool areas with higher dissolved oxygen, even if the temperatures were a bit higher as well (Matthews and Berg 1997). It could be that the combination of biological oxygen demand related to the decomposition of the muck, in addition to the higher water temperatures, reached a stress threshold that proved to be too great for not only the *O. mykiss*, but also the other fish species and crayfish.

In 2007 and 2008 "Malibu muck" re-appeared and remains an element in the benthic zone. The possible influx of a transient toxin or pollutant cannot be totally discounted, and the continued presence of the muck indicates that if eutrophication contributed to the 2006 decline, the threat still remains. There is also concern that a pathogen associated with the diatom community could be a factor in the die-off, as was noted in Sycamore Creek, Arizona, where invasive bacteria caused a massive die-off of the benthic diatom community (Peterson and Dudley 1995).

The die-off was a sobering reminder of how quickly a small population can be extinguished and reinforces the need to monitor and preserve all geographic populations of southern steelhead. It also points to the need to have a rapid response plan in hand, in case such an event should occur again. To that end, the following protocol has been developed and permits are being obtained to facilitate a more coordinated and rapid response to observation of discolored or sick fish in the future.

Should trout be observed with the yellow coloration, sluggish swimming, gaping and/or other evidence of disease or illness, the following steps will be taken:

1. Notify NMFS and CDFG immediately and invite agency staff to confirm the problem.
2. Document conditions with video if possible (visibility can be a limiting factor) and send images to NMFS, CDFG pathologists for review.
3. Convene the ad-hoc Technical Advisory Committee (TAC) described above, and add additional expertise if needed.
4. Initiate continuous year round water quality monitoring to include, but not be limited to: temperature, dissolved oxygen, pH, nutrients, heavy metals, pesticides and toxicity. (A Troll 9500 probe is being installed near lower twin pool in 2008 to collect temperature, dissolved oxygen, conductivity, pH and pressure)
5. Check for acute pollution event, in collaboration with Las Virgenes Municipal Water District, Heal the Bay, Malibu Creek State Park, Los Angeles County Vector Control and the Los Angeles Regional Water Quality Control Board.
6. Evaluate in-stream conditions regarding muck and algal decomposition. Collect samples for examination of species composition, bacteria and toxicity in collaboration with UC Santa Barbara, Cal State San Marcos, UCLA.
7. Collect specimens of other fish species and crayfish that exhibit signs of stress or illness for examination by the CDFG pathologists.
8. Continue examination of other accounts of yellow morphology in trout.
9. Collect specimens of both native and New Zealand mud snails for examination for trematodes.
10. If conditions warrant, collect one – three affected *O. mykiss*, under the direct supervision of NMFS and CDFG pathologists, for both live examination and post-mortem histology and pathology testing.

11. If conditions warrant, install secured live car cages in the creek containing triploid, sterile, disease free hatchery rainbow trout to test survival and further infection potential, under the direct supervision of CDFG pathologists.

It is hoped that this die-off was an isolated incident and the recovery of both *O. mykiss* and other fish species in 2008 has demonstrated that an area can re-populate quickly following a traumatic event. Given the precarious status of *O. mykiss* in Southern California, we need to develop regional strategies for rapid response should this kind of event occur again.

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Gonadal Restructuring During Sex Transition in California Sheephead: a Reclassification Three Decades After Initial Studies

Michael A. Sundberg, Kerri A. Loke, Christopher G. Lowe,* and Kelly A. Young

Department of Biological Sciences, California State University Long Beach, 1250 Bellflower Blvd., Long Beach, California, 90840, USA

Abstract.—California Sheephead, *Semicossyphus pulcher*, is a monandric protogynous hermaphrodite and a commercially and recreationally valuable labrid. Gonadal functionality of Sheephead through sex change was reclassified into nine classes using current criteria for categorization. Female ovaries were classified as immature, early maturing, mature, and regressing/recovering classes. Transition from female to male and subsequent male development was divided into early, mid and late transitional, developing/active male and regressing/recovering male. Reproductive states in Sheephead were correlated with estradiol (E2) and 11-keto testosterone (11-KT) concentrations in the blood plasma. All sexes had low E2 concentrations in the fall /winter seasons; in transitional and male individuals, levels remained low throughout the year. In contrast, female E2 concentrations were elevated in spring and peaked in the summer. Concentrations of 11-KT were variable throughout the year; however, females had significantly lower levels in the summer. This study allows a better understanding of the current state of California Sheephead in a heavily fished area. Knowledge of a species' reproductive characteristics is important in evaluating the sustainability of a population as it can set a baseline for reproductive potential. This research takes a critical step in gathering and organizing reproductive data such that it may be used in future studies for comparing reproductive potential across the range of the California sheephead.

Introduction

Over the last 50 years, research efforts have focused on the process of post-maturational sex changes in fishes. This process has been described by histological studies of the gonads, as well as through characterization of body morphology (Moe 1969; Choat and Robertson 1975; Warner 1975; Shapiro 1981, 1987; Ross 1987; Hastings 1989; Sadovy and Colin 1995; Bruslé-Sicard and Fourcault 1997; Liu and Sadovy 2004; Mackie 2006). The mechanism and direction by which different fishes change sex varies significantly (Munday et al. 2006), making a descriptive analysis of sex change characteristics and methods for quantifying sex change both fundamental and foundational for further research of any particular hermaphroditic species.

California Sheephead, *Semicossyphus pulcher* (Ayres), is a commercially and recreationally valuable labrid found from Monterey Bay, California, to Cabo San Lucas, Mexico (Miller and Lea 1972). Sheephead are an epibenthic species that generally utilize sand-rock reef habitats for foraging while taking shelter and reproducing in and over rocky reefs habitat (Topping et al. 2005). Because adult *S. pulcher* feed heavily on

*Address Correspondence To: Christopher G. Lowe, Department of Biological Sciences, California State University Long Beach, 1220 Bellflower Blvd, Long Beach, CA 90840, clowe@csulb.edu

urchins, they are an important species for indirectly regulating kelp growth in southern California's coastal waters (Cowen 1983, Dayton et al. 1998). Overfishing has resulted in depletion of many populations of Sheephead throughout southern California (Alonzo et al. 2004; Hamilton et al. 2007).

As monandric, protogynous hermaphrodites, California Sheephead can transition from a reproductively functional female to a functional male during the course of a lifespan in response to social factors (Warner 1975; Adreani et al. 2004). The transitional phase has been reported in other sex-changing teleosts to take from 2–3 weeks to several months (Robertson 1972; Mackie 2003; Sadovy and Shapiro 1987; McBride and Johnson 2006). While sex hormones have not been previously examined during transition in Sheephead, we expect that changes in steroid hormone concentrations; specifically, 17β-estradiol (E2) and 11-ketotestosterone (11-KT) are related to sex change due to the total degradation of the ovaries and the appearance of testes.

Although ambisexuality is widely displayed in teleost fishes and there have been numerous studies describing the sex change process in fishes, comparisons within and among species have been limited due to inconsistencies in gonad class description and terminology. Recently, proposals for a classification system of gonad development in both gonochoristic and hermaphroditic fishes have been made (e.g., Brown-Peterson 2006; Barbieri et al. 2006; Brulé and Colás-Marrufo 2006). To reflect the growing body of scientific knowledge, and to provide consistency among studies, it is important that attempts be made to bring descriptions of fish sexual development in line with more current terminology.

Reproduction in California Sheephead was first described more than 30 years ago using histological methods, and as individuals change from female to transitional to male, several classes of development were identified (Warner 1975). This study re-examines Sheephead gonadal development in light of several decades of environmental and anthropogenic pressures, with the goal to update Sheephead gonadal development descriptions to aid in future studies of the sex-change process within this species. In addition, we sought to correlate changes in the gonads with alterations in plasma concentrations of sex steroid hormones. Strong correlations between sex hormones and gonad state could provide a non-lethal means to determine reproductive state and this may be a useful tool for both future researchers and fishery managers.

Due to their economic significance and population decline, a comprehensive and current model of Sheephead reproductive function at the gonadal level is critical for proper future management and research of the species. Such a model could be valuable in determining the health of the Sheephead population, in terms of reproductive capacity. In this paper, we identify and reclassify the reproductive classes of Sheephead caught near Santa Catalina Island, California, using current terminology. In doing so, we take the first steps in determining the current reproductive profile of the Santa Catalina Island Sheephead population.

Materials and Methods

Animals

California Sheephead were obtained within a 2 km radius of Bird Rock, Santa Catalina Island, California (33°29′N, 118°27′W) from October 2004 through October 2005. Fish were caught by hook and line at depths ranging from 5 to 40 m, or by baited traps at depths of 14 to 21 m. Once caught, fish were brought to the surface and their swim bladders were vented with a hypodermic needle to relieve the effects of barotrauma.

Sheephead were then transported in a 60 L cooler to the laboratory for dissection. The maximum time between capture and dissection was 5 hours. Each fish was euthanized in an ice-slurry immediately prior to dissection.

Each fish was immediately assigned to one of three gender groups (female, transitional, male) based on previously described external morphological characteristics and coloration (Warner 1975). In addition, blood was collected via the caudal vein prior to euthanization to assess the seasonal relationship of certain steroid hormones (E2 and 11-KT) to gonad function. Blood samples were centrifuged at 1500 rotations per minute for 5 minutes and blood plasma was removed. Plasma samples were stored at −80°C until collections were complete and hormone assays could be conducted.

Tissue Processing

After euthanization, bilobate gonads were excised, cleaned of connective tissue and weighed. Portions from each end and the midsection of the gonad lobes were immersed in 10% formalin to maintain protein structure and composition. Gonads were then processed for histological analysis.

After 10 days in formalin, gonads were washed in phosphate-buffered saline solution (PBS), dehydrated in a graded series of ethanol solutions, and embedded into paraffin. Gonads from each individual were cut in 6 μm thick sections and mounted onto slides. Sections from each gonad were mounted serially with 60 μm of tissue between each mounted section. All sections were mounted onto Superfrost-plus microscope slides (Fisher Scientific, Pittsburgh, PA). Sections were placed through a series of xylene and ethanol washes in order to hydrate the tissues, and stained using hematoxylin and eosin.

Gondal Tissue Characterization

Female, male and transitional gonadal tissues were identified and characterized based on previous work (Warner 1975; Guraya 1986; Sadovy and Shapiro 1987; Nakamura et al. 1989; Taylor et al. 1998), and recent efforts to standardize terminology in describing gonad tissue development were consulted (e.g., Brown-Peterson 2006; Barbieri et al. 2006; Brulé and Colás-Marrufo 2006). The terms "class" and "stage" were used to define the development of the gonad and the gametes, respectively.

Follicle Counts and Characterization of Transitionals/Males

Serial sections of female gonads were used to analyze the relative density of ovarian structures. Class of gonadal development was recorded using brightfield microscopy, and the number of structures for each follicle stage within or overlapped by a 1 mm^2 guide was counted. Three arbitrarily selected locations within each tissue section were counted. For each ovary, the average relative density of each ovarian structure per section was determined from the six representative gonadal cross sections counted per individual.

Transitional individuals and males were assessed by observing both the level of gonadal reconstruction (degeneration of ovarian structures and generation of testicular tissue) and the stage of spermatogenesis. Determination of sex was made based on the most predominate stage of spermatogenesis observed in the six representative sections of gonad for each animal.

Hormone Analysis

Estradiol concentrations in the blood plasma were measured using DSL-4800 Ultra-Sensitive Estradiol RIA ^{125}I double antibody kits (Diagnostic Systems Laboratories, Inc.,

Webster, TX). Samples were assayed in duplicate and radioactivity was measured using a Perkin-Cobra II gamma counter (Packard Instruments Co., Boston, MA). SigmaPlot 8.0 software (SPSS Inc., Chicago, IL) was used to generate a standard curve in the four-parameter logistic curve function and determine hormone concentrations. Assay standards and controls were within the normal limits (Moffatt-Blue et al. 2006; Schmidt and Kelley 2001), with a 2.2 pg/mL lower limit of detection and low (0.64–2.40%) cross-reactions to other steroids.

Plasma 11-KT concentrations were measured using competitive enzyme immunoassays kits (ACE^{TM} kits from Cayman Chemical Co., Ann Arbor, MI). Each sample was assayed in duplicate and two dilutions of each sample with between 20 and 80% B/B0 values were averaged. Plates were read using a Powerwave XS Bio-Tek microplate spectrophotometer at 412 nm. Raw data (absorbances) were exported to Microsoft Excel spreadsheets and analyzed using 2006 Cayman Chemical Enzyme Immunoassay (EIA) Tools software, (Cayman Chemical Co.). The average intra-assay variability was 8.3% and the lower limit of detection was 5.3 pg/mL.

Data Analysis

For most analyses, fish were grouped according to the season in which they were caught; *fall* (October through December), *winter* (January through March), *spring* (April through June), and *summer* (July through September). Changes in seasonal follicle densities were analyzed using ANOVA analysis. A Student-Newman-Keuls post-hoc test was used to compare differences in follicle densities between seasons ($\alpha = 0.05$). Seasonal E2 concentrations were analyzed using a Friedman's two-way non-parametric ANOVA ($\alpha = 0.05$) to compare effects of season and gender. Kruskal-Wallis tests were used post-hoc to compare differences in E2 levels among sexes by season, and a Mann-Whitney test was used to compare differences among females across seasons. Concentrations of 11-KT for females, transitional individuals and males were square-root transformed and a two-way ANOVA ($\alpha = 0.05$) was used to assess differences.

Results

Catch Assessment

A total of 133 California Sheephead were caught over the course of a year at Santa Catalina Island, of which 94 adult fish were used in the histological analysis. Based on observations from these mature individuals, as well as several immature gonads, the development from female to male was characterized on a continuous scale ranging over nine gonad development classes (Table 1).

Ovarian Morphology and Seasonal Changes

Female gonad development comprised the first four of the nine classes, and determination of gonad class was made using the predominant oocyte structural stages present in the ovary (Figure 1). Female ovaries were classified as *immature* (Figure 1A), *early maturing* (Figure 1B), *mature* (Figure 1C), and *regressing/recovering* (Figure 1D) classes. The *regressing/recovering* ovaries of females not transitioning to males return to the *early maturing* class. Oocytes move through four stages of development before release during spawning (*chromatin nucleolar oocytes*, *perinucleolar oocytes*, *yolk vesicle oocytes*, *yolk globular oocytes*), whereas follicles with unreleased oocytes underwent atresia (Figure 1D) within the ovary. Post-ovulatory follicles were also noted in gonads found in the mature ovary class (Figure 1C).

Table 1. Reproductive classification of the protogynous hermaphrodite, California Sheephead (*S. pulcher*). Reproductive class is based on gonad morphology and histological examination of gonad structures.

Sex	Reproductive class	Gonad characteristics
Female	1. Immature	Gonads contain primary growth stage oocytes only, including chromatin nucleolar oocytes (CO) and perinucleolar oocytes (PO). Ovarian walls are thin and lamellae are tightly packed. Cytoplasm is generally basophilic and stains darker than more mature cytoplasm.
	2. Early maturing	Yolk vesicle (cortical alveolar) oocytes (YVO) are notable, along with CO and PO. As follicles begin maturing, they become less basophilic and appear slightly lighter in coloration to surrounding primary growth oocytes.
	3. Mature	Yolk globular oocytes (YGO) are present along with all other follicular development stages. Post-ovulatory follicles (POF) are also present. Follicular cytoplasm is relatively acidophilic and stains much lighter than previous stages.
	4. Regressing/recovering	Many CO and PO are present, along with widespread atresia of residual YVO and YGO.
Transitional	5. Early transitional	A high level of atresia and a high disruption of tissues. Some CO and PO are present. Few to no spermatocysts notable. Few specimens were noted in this stage.
	6. Mid transitional	Some CO and PO still present, along with many atretic follicles. Several spermatocysts (SC) of earlier stages in spermiogenesis are notable in the lamellae, however few spermatids and maturing sperm.
	7. Late transitional	CO and PO no longer present, however pockets of large atretic follicles are still notable. SC have become more numerous and may contain all stages of spermatogenesis. The tissue is primarily testis.
Male	8. Developing/Active	SC during developmental stages become dense and numerous, with all stages of spermatogenesis potentially present. As development continues males become reproductively active; spermatids (ST) and spermatozoa (SZ) become more prevalent. Few to no atretic follicles remain in the gonads.
	9. Regressing/recovering	Residual spermatocysts (RSC) remain containing spermatozoa. Primary and secondary spermatocytes (1SY and 2SY) are most prevalent spermatogenic stage, and some SC containing 1SY can also be observed.

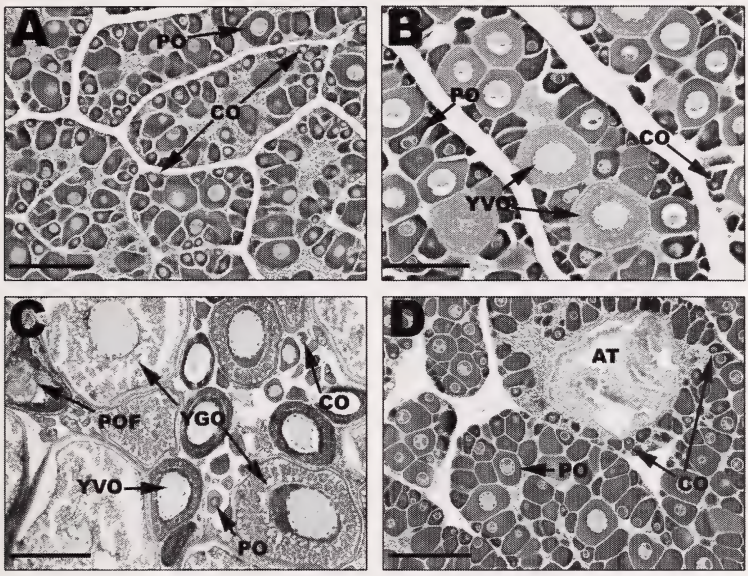

Fig. 1. Representative photographs of female California Sheephead ovarian follicle development. Bars in the lower left corner of each photo represent 200 micrometers. (A) Immature female gonads. (B) Maturing ovaries. (C) Mature ovaries. (D) Regressing/recovering ovaries. AT = atretic follicle. CO = chromatin nucleolar oocyte. PO = perinucleolar occyte. POF = post-ovulatory follicle. YVO = yolk vesicle oocyte. YGO = yolk granule oocyte.

Histological analysis of gonads throughout the year showed seasonal changes within the structure of female gonads (Figure 1). During the winter season, adult females exhibited only primary growth oocytes (chromatin nucleolar oocytes and perinucleolar oocytes) and atretic follicles (Figure 1D). Follicle development (ovarian follicle stage densities – yolk globular oocytes and atretic follicles) differed across seasons (Kruskal-Wallis: $H = 17.71$, d.f. = 3, $p = 0.001$; Figure 2A; Kruskal-Wallis: $H = 10.50$, d.f. = 3, $p = 0.015$; Figure 2B). Atretic follicle count data were normalized by adding 0.1 to all values and log transforming. Oocyte development became evident during the spring and summer months and peaked in the summer, with relative mean density of summer yolk globular oocytes 8.1-fold greater than in spring (Figure 2A). No yolk globular oocytes were observed in winter samples. The densities of atretic follicles peaked in the fall and winter and were lowest in the summer (Figure 2B).

Morphological Development and Seasonal Changes of Testes

Gonadal transition from female to male and subsequent male development was divided into five classes, with the first three classes considered transitional. During the early stages of sexual transition, chromatin nucleolar oocytes, perinucleolar oocytes, and atretic follicles were observed within the tissue (Figure 3A), along with developing spermatocysts that became more common in mid-transitional fish (Figure 3B). In early transitional individuals, the gonads were greatly reduced in size. In late transitional individuals, testicular tissue was more prevalent and later stages of spermatogenesis were noted in some specimens, including primary and secondary spermatagonia, primary and secondary spermatocytes, spermatids and occasionally spermatozoa (Figure 3C). The presence of these cell types indicate that fish in late transition may be reproductively

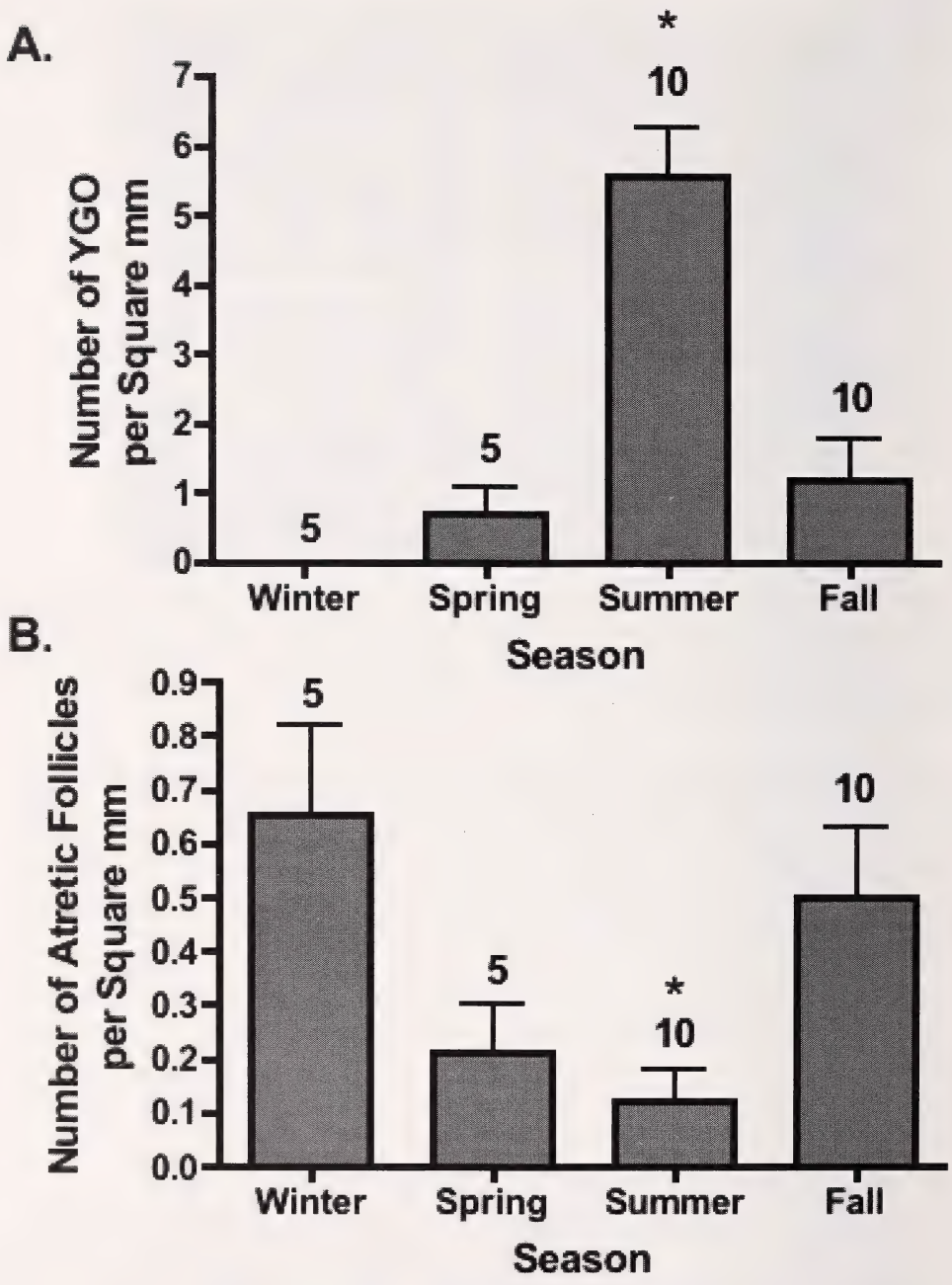

Fig. 2. (A) Mean density of yolk-globular oocytes (cells per mm^2) for mature females over seasons; (*) represents significant difference from all other groups. (B) Mean density of atretic follicles (cells per mm^2) for mature females over seasons; (*) represents significant difference from winter and fall. Error bars represent one Standard Error. Numbers above each bar represent the sample size.

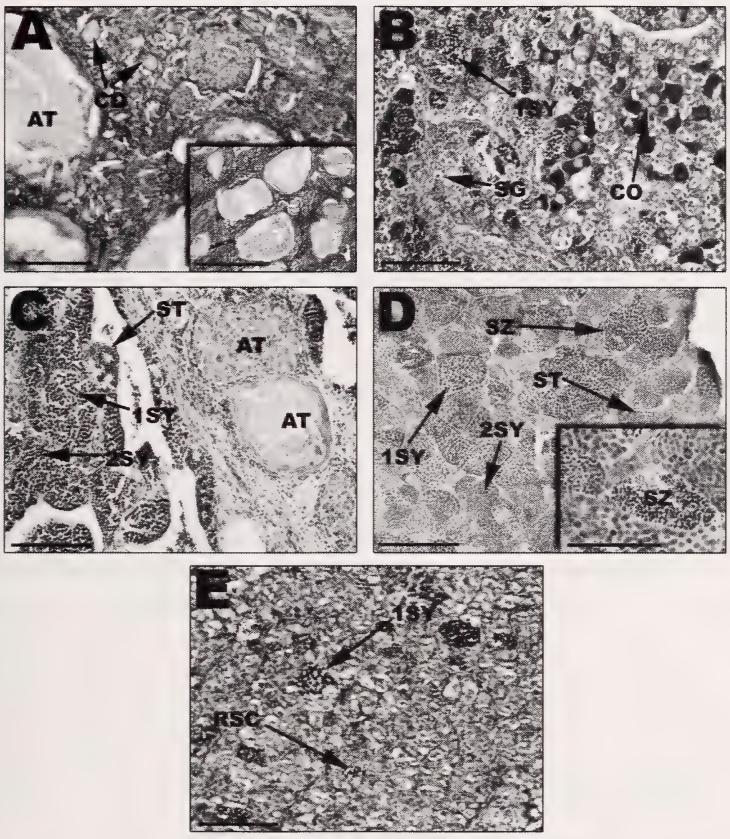

Fig. 3. Representative photographs of male California Sheephead testicular development. Bars in the lower left corner of each photo represent 100 micrometers. Inset bars in (A) and (D) represent 400 micrometers and 50 micrometers, respectively. (A) During the earliest noted transition class very high levels of atretic follicles are noted, few early stage oocytes, and massive tissue reorganization. (B) As the transition progresses, spermatogonia and primary spermatocytes can be observed among early stage oocytes. (C) In late transition, early stage oocytes are very rare, with further stages of spermatogenesis noted. (D) Active males commonly exhibit the final stages of spermatogenesis. (E) Inactive males contain mostly residual spermatocytes and early stage spermatogenic tissue. AT = atretic follicle. CO = chromatin nucleolar oocyte. RSG = residual spermatocyte. SG = spermatagonia. ST = spermatid. 1SY = primary spermatocyte. 2SY = secondary spermatocyte. SZ = spermatozoa.

functional as male. In addition, primary stage oocytes were no longer found in the gonads of fish classified as late transitional, although oocyte atresia was still apparent (Figure 3C). Gonads of fish classified as mature males showed no residual atretic follicles (Figure 3D). During the summer breeding months, the male gonad was characterized by dense spermatocysts of all developmental stages (e.g., Figure 3D); while during the winter non-breeding months the mature male testis contained mainly spermatogonia, residual spermatozoa, and some primary spermatocytes (Figure 3E).

Hormone Concentrations

No measurable levels of E2 were detected in most transitional individuals or in males. Concentrations of E2 differed significantly by season ($F_{3,90} = 7.62$, $p = 0.0001$) and by gender ($F_{2,90} = p < 0.0001$; Figure 4A). There was no difference in E2 concentrations

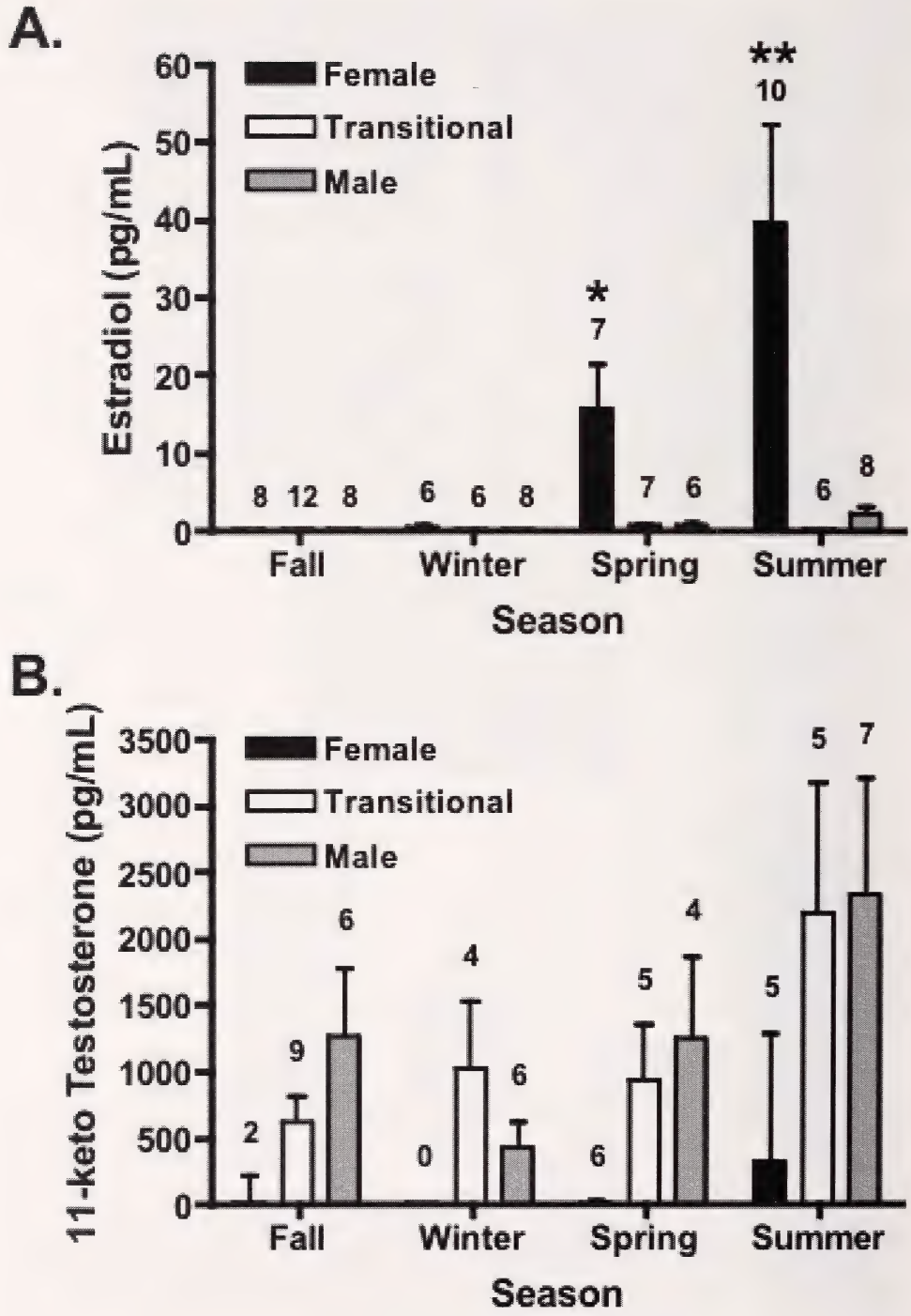

Fig. 4. Mean seasonal plasma E2 (A) and 11-KT (B) concentrations of female, transitional and male Sheephead at Santa Catalina Island. Error bars represent one Standard Error and (*) represents significant difference. Numbers above each bar represent the sample size.

among genders in fall (Kruskal-Wallis: $H = 0.00$, d.f. $= 2$, $p = 1.0$) and winter seasons (Kruskal-Wallis: $H = 2.33$, d.f. $= 2$, $p = 0.31$); however, females had significantly greater E2 levels than transitional individuals and males in spring (Kruskal-Wallis: $H = 9.7$, d.f. $= 2$, $p = 0.008$) and in summer (Kruskal-Wallis: $H = 15.76$, d.f. $= 2$, $p = 0.000$). Female E2 concentrations peaked in the summer and levels were significantly greater in the summer than in the spring (Mann-Whitney: $W = 42.5$, $p = 0.05$).

There were significant differences in 11-KT by gender ($F_{2,60} = 17.5$, $p < 0.001$) and season ($F_{3,60} = 0.033$, $p = 0.033$; Figure 4B). Concentrations of 11-KT were significantly higher in males (Tukey: $p < 0.001$) and transitionals (Tukey: $p < 0.001$) than females overall; however, only during the summer season (Tukey: $p = 0.049$).

Discussion

Populations of California Sheephead have been significantly reduced due to overfishing and this decline has resulted in a reduction in the size at transition, an altered sex ratio, and likely an increase in the rate of transition (Hamilton et al. 2007). The current data present an updated assessment of California Sheephead protogynous sexual development within the context of these recently reported changes in population structure and are intended for use in future studies of California Sheephead populations and other temperate teleost species. We have established and updated the definition of female, transitional, and male gonad classes concomitant with the current and revised gonadal terminology. Critically, these classifications reveal changes that have occurred in the Santa Catalina Island population that mirror recently reported alterations in population structure at other heavily fished islands (Hamilton et al. 2007). Although we cannot determine from this study the time required to change sex or the impact on the overall reproductive capacity, our findings indicate that there is a period of reproductive inactivity involved during gonadal remodeling. Future studies should examine the effects of changing sex related to the timing of sex change on the reproductive capacity of Sheephead at the individual and population level.

A recent consortium for uniformity and consistency in the classification of teleost gonad structure has sought to reduce the discrepancies present across histological studies of different fishes (e.g., Nunez and Duponchelle 2009; Brown-Peterson 2006; Barbieri et al. 2006; Brulé and Colás-Marrufo 2006). While the refined terminology was originally predicated upon non-hermaphroditic species, we have applied the current terminology to the sexual development and transition of California Sheephead gonads. The current study categorized sex change into nine classes, as opposed to the prior eight (Warner 1975). The sample sizes of transitional individuals in the present study exceed what was observed previously, allowing the classification of two more transitional classes, while deleting one male (post-spawning) class as it was never observed during this study and is thought to be a relatively short-lived phase.

Our histological data suggests that sex change in Sheephead is unidirectional. While the exact timing of transition from functional female to functional male remains unclear, ovarian remnants in later stage transitionals are atretic as compared to ovarian structures with further developmental potential (chromatin nucleolar oocytes, perinucleolar oocytes, and mature oocytes) found in males of other fish taxa known to exhibit bi-directional sex change (Cole 2003; Wittenrich and Munday 2005). It is possible that in the later stages of the transition, fish are functionally male but maintain an intersexual gonadal appearance (Sadovy de Mitcheson and Liu, 2008). Among late transitional fish, the presence of later stages of spermatogenesis along with ovarian remnants is an

important criterion for determining fish still within the transitional classes (Sadovy and Shapiro 1987). Behavioral and further histological studies must be coordinated to determine if mature sperm are released during mating among Sheephead that exhibit these gonadal characteristics. Among early and mid- transitional fish, where ovarian tissue persists and still includes oocytes in early developmental stages and spermatogenic tissue remains sparse, the potential to function as a male remains unknown but is likely far lower than those in the late transitional stage. Categorizing transition from female to male into three classes as opposed to leaving it as one (Warner 1975) allows for better future estimates of the reproductively non-active percentage of a Sheephead population, especially for those in transition during the breeding season.

Concentrations of E2 were elevated in spring and peaked among females in the summer as compared to the non-breeding season months; levels were not significantly different from males or transitionals in the fall and winter months (Figure 4). This seasonal peak corresponds with maximal ovarian function as observed in mature gonads from summer fish. However, it appears that females begin to prepare for the breeding season in late spring because ovarian function (i.e. E2 concentrations and yolk globular oocytes) is higher in spring than in fall and winter (Figure 1). During spring and summer, concentrations of E2 were significantly higher in females as compared to both males and transitionals; for transitionals this held true regardless of transition class. This suggests that follicular function, as measured by E2 production, declines rapidly as females undergo transition. Indeed, atretic follicles were observed in all classes of transitioning fish: early, mid, and late. Interestingly, the decline in plasma E2 among transitional fish as compared to females was observed during the breeding as well as the non-breeding season. Together, these data suggest that once an individual initiates sex change, there is a rapid and lasting decline in female gonadal function.

While 11-KT appeared to peak in the summer breeding season as opposed to non-breeding months these increases were not significant (Figure 4). Typically, 11-KT is the primary androgen in male teleosts, and generally is highest concomitant with the breeding season to facilitate reproductive behaviors and physiology (Borg 1994). In California Sheephead, however, 11-KT concentrations were widely variable, without a clear summer peak in males. It may be that the pulsatile nature of this hormone resulted in the high variability observed across males; however, the lack of a significant peak during the breeding season may also suggest that other androgens are playing a larger role in reproduction in this species.

In summary, these data show for the first time that at the heavily-fished Santa Catalina Island Sheephead population, gonadal function corresponds to peak hormone function, particularly in females. In light of the substantial changes in population structure at Santa Catalina Island as recently reported for Sheephead (Hamilton et al. 2007), we have reexamined the classification of gonadal morphology and updated terminology. Our findings will allow for better comparisons of reproductive capacity across Sheephead populations. Our endocrine data suggest that hormone analysis may provide a non-lethal method of wide-scale sampling for Sheephead. Together, these methods may be used across the Sheepheads range to determine variations is social structures of different populations and possibly better understand the effects of sex biased fishing pressure on these populations.

Acknowledgments

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Surface-dwelling and Subterranean Invertebrate Fauna Associated with Giant Reed (*Arundo donax* Poaceae) in Southern California

Robert E. Lovich,[1] Edward L. Ervin,[2] and Robert N. Fisher

U. S. Geological Survey, Biological Resource Discipline, Western Ecological Research Center, San Diego Field Station, 4165 Spruance Rd., Suite 200, San Diego, CA 92101-0812, USA

Abstract.—In the southwestern United States giant reed, *Arundo donax*, is a non-native invasive plant that has become widely established in moist places and forms its largest stands along riparian corridors. The most widely reported negative effects include competition with native species, increased rate of transpiration, increased potential for wildfires, and stream channel and bank alteration. However, little is known about the faunal communities associated with this plant and the potential effects on native fauna. In this study, we focused our efforts on determining the faunal composition specifically from rhizome clumps of *A. donax* from a site located along the Santa Margarita River in San Diego County, California. A total of 2590 individual macro-invertebrates were collected and identified, and represented 64 species from 7 classes. No sensitive species and few vertebrates were found to be in association with *A. donax* rhizome clumps. Four non-native invertebrate species made up 43% of the total number of captured invertebrates, and 31% of the sampled invertebrates were confirmed as native species. This study demonstrates that *A. donax* rhizome clumps, and the soils associated with them, provide habitat for several native macro-invertebrate species, but can be dominated by a greater abundance of non-native species.

Giant reed, *Arundo donax*, is a non-native invasive species from the Old World that has become widely established throughout riparian habitats in southern California (Dudley 2000). Previous studies have shown that *A. donax* competitively displaces native vegetation (Rieger and Kreager 1989), transpires greater quantities of water than native vegetation (Iverson 1994), increases the potential for wildfires (Bell, 1994), creates biological pollution by contributing vast amounts of detritus (Douce 1994), and alters channel and bank morphology (The Nature Conservancy 1996). However, given the dramatic changes in vegetation diversity and structure, hydrology, channel morphology, and fire regime that results from the invasion and establishment of *A. donax*, relatively little has been reported on the fauna associated with large clusters of *A. donax* thickets (Herrera 1997; Herrera and Dudley 2004).

Critical to the management of *Arundo donax* is a thorough understanding of the ecological relationships between this invasive plant and other species. This is especially important in southern California where critical populations of at-risk species are reliant

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[1] Current address, Department of Earth and Biological Sciences, Loma Linda University, Loma Linda, CA, 92350; rlovich@gmail.com

[2] Current address, Merkel & Associates, Inc., 5434 Ruffin Road, San Diego, CA 92123

Table 1. Physical characteristics of 41 rhizome clumps.

	Length (m)	Width (m)	Depth (m)	Volume	Percent rhizome clumps	Percent dirt	# of Species per rhizome clumps	# of Indiv. per rhizome clumps
AVERAGE	3.49	2.28	0.54	1.06	19.13	80.32	16.4	63.0
STANDARD DEVIATION	0.71	0.2	0.9	0.37	12.67	12.72	5.8	40.7

on the small and sometimes isolated remaining riparian and wetland ecosystems that still exist (Dudley and Collins 1995). The primary objective of this study is to determine the subterranean and surface-dwelling fauna that occur in *Arundo donax*.

Materials and Methods

The study site was located along the Santa Margarita River at Marine Corps Base Camp Pendleton (hereafter, MCBCP). The site was located in the interior of a several hectare infestation of *A. donax*. Sampling was accomplished by first snapping off the vertical stalks of the *A. donax* at their base by mechanically sweeping a grappler extension close to the ground. Following the clearing of an area, the grappler would grab and stack clipped plants into large piles. The grappler would periodically transfer these piles to large dump trucks for removal. Within the study area, specific locations for the mechanical grab of each *A. donax* rhizome clump was opportunistically selected by biologists trying to sample multiple sites to capture minor variations in habitat conditions (e.g. depth of leaf litter, % canopy cover) of the dense monotypic stand. Each grab by mechanical grappler extracted a single *A. donax* rhizome clump. Each rhizome clump was immediately placed upon a tarp following extrication and was immediately broken apart and carefully inspected for fauna. Clumps of *A. donax* were manually broken apart by hand or with the aid of hand tools (i.e., pickaxe, shovel, steel rake, sledge hammer and claw hammer). All macro-invertebrate taxa were collected, sorted, and immediately stored in 70% ethanol. In addition to manually sorting through the clump, random samples of associated sediment were sieved and stored in 70% ethanol to collect any specimens not previously located by the initial visual search. The inspected rhizome clumps were then set aside into a pile adjacent to the soil from the same clump; the relative volumes of rhizome clumps and soil piles for each clump were then visually estimated. Physical characteristics including length, width, and maximum depth of the hole created by the mechanical removal of each clump are provided in Table 1. A total of 41 clumps were surveyed between 28 Sept. 2000 and 24 Oct. 2000. All specimens collected during the field portion of the study were keyed out to morphospecies.

Initial sorting of collected macroinvertebrates utilized parataxonomists (personnel not formally trained in taxonomy). All collected specimens were initially sorted into one of 140 identified morphospecies classes. Morphospecies classes were determined by obvious visually identifiable differences. Following the sorting by non-specialists, samples were then transferred to invertebrate taxonomy specialists and keyed out. A determination of whether species were native or non-native was also made for as many of the sampled taxa as possible. All specimens are housed at the United States Geological Survey, San Diego Field Station, San Diego, California, USA.

Table 2. Number of species identified by class.

Annelida	1
Arachnida	23
Chilopoda	2
Crustacea	2
Diplopoda	2
Insecta	33
Molusca	1

Results

Forty-one rhizome clumps were examined between 28 September 2000 and 24 October 2000. Average size of each rhizome clumps was 1.06 m^3, as calculated from length, width, and depth of the hole created by removal of rhizome clumps, with physical characteristics summarized in Table 1. A total of 2590 individual macroinvertebrates were collected and identified, representing 64 species from 7 classes (Table 2). Four non-native species made up 43% of total captured arthropods *Armadillium vulgare* (pillbug), *Porcellio laevis* (sowbug), and *Dysdera crocata* (sowbug killer), and *Linepithema humile* (Argentine ant). Thirty one percent of all sampled invertebrates were confirmed as native, with spirobolid millipedes making up over 11% of total captures. Over 55 species were represented by 3 individuals or less. Relatively low densities of invertebrates were found under the clumps and living in and around the rhizome clumps that were examined. In 32 of the 41 rhizome clumps, less than 100 individual invertebrates were found. On average there were 16 species and 63 individuals found per rhizome clump (Table 1). Fifty six of sixty four species of invertebrates sampled were from two classes, Arachnida and Insecta (Table 2). None of the other five classes were represented by more than 2 species.

Diversity of all sampled invertebrates (n=2590), as measured by the Shannon Wiener index (Krebs 1989) was 1.28, or about 71% of maximum possible diversity or evenness (1.80) assuming an equitable distribution of all taxa across the sampling effort (Table 3). Those species that were confirmed as native (n=901, 29 species) had a diversity of 1.03, or 71% of maximum possible diversity (1.46), while confirmed non-native species (n=1201, 8 species) had a diversity measure of 0.62, or 68% of maximum possible diversity (0.90, Table 3). There were 488 specimens that could not be determined as native or introduced, because they could not be keyed out to species and/or so little is known about their distribution, and which composed at least 27 distinct taxa. The native and non-native Shannon Wiener diversity indices were tested for the null hypothesis that these two diversities are equal following the two-tailed t test of Hutcheson (1970), as outlined in Zar (1984). The null hypothesis was rejected ($t = 10.51$, $df = 996.0$, $p < 0.001$), and native species diversity is significantly greater than non-native species diversity.

Table 3. Diversity indices for all captures, confirmed native, and confirmed non-native species.

	Total Species (n=2590)	Native Species (n=901)	Non-native Species (n=1201)
SHANNON DIVERSITY (H′)	1.28	1.03	0.62
SHANNON EVENNESS MEASURE (J′)	0.71	0.71	0.68
MAXIMUM DIVERSITY (H′max)	1.81	1.46	0.90

Few vertebrates were observed, but the vole (*Microtus californicus*) (n=4, 2 live, 2 dead), woodrats (*Neotoma sp.*) (n=2, live), and the Pacific treefrog (*Pseudacris hypochondriaca*) were observed. Black-tailed deer (*Odocoileus hemionus*) and western fence lizards (*Sceloporus occidentalis*) were also sighted along the edge of the *A. donax* thickets being removed.

Discussion

Non-native species comprised at least 45% of all collected invertebrates in *A. donax*, while confirmed native species represented 31% of all collected invertebrates. 24% of the collected individuals could not be assigned as native or non-native due to lack in basic distribution data for these species. This indicates some variation in the use of *A. donax* by native versus non-native species, with non-native species being more abundant. Four species made up 97% of all confirmed non-natives from the samples. Shannon Wiener diversity measures of natives versus non-natives also differ significantly when tested ($t = 10.51$, $df = 996$, $p < 0.001$). Non-native species were more abundant, and diversity measures reflect this. Diversity indices for these two groups differ statistically, and indicate that native species have a significantly higher diversity than non-native species. Confirmed native species are also represented by 29 species, versus 8 non-native species. It is interesting that the diversity of native species is greater than that of non-native species in an entirely non-native stand of vegetation, but both have similarly high levels of evenness (71% for natives, and 68% for non-natives). This indicates that many native species are found in association with *A. donax*, but clearly, there are large numbers of only a few non-native species.

Figure 1 shows the number of individuals captured for each species, and highlights the three most abundant species. This finding is important to understanding the role and impacts of *A. donax* to native species in the riparian habitats it occupies. Species sampled in this study were largely ground dwelling invertebrates, which have been shown to differ less in diversity across non-native habitats than their aerial counterparts (Herrera and Dudley 2004). Other studies have shown that invertebrate abundance and diversity declined in non-native habitats when compared with those in adjacent native habitats (Slobodchikoff and Doyen 1977; and Beerling and Dawah 1993).

It is well known that non-native species pose a considerable threat to the biodiversity of California (Mooney et al. 1986). A previous study at Sonoma Creek California (Herrera 1997; Herrera and Dudley 2004) sampled both aerial and ground-dwelling invertebrates in *A. donax* dominated areas, mixed Arundo- native willow, and willow riparian habitats. Their results found that native insect abundance, biomass, and species richness were significantly higher in native riparian vegetation than in *A. donax* dominated riparian habitat. Biomass was not analyzed in this study, but confirmed native insects were less abundant than confirmed non-natives (Fig. 1). Herrera and Dudley (2004) found that abundance and diversity declined from spring to summer for invertebrates in *A. donax*, and Bolger et al. (2000) found that diversity and abundance of arthropods were lowest in the fall. Our study gathered results only during the fall, so seasonal comparisons cannot be made.

It is well known that large numbers of invertebrates have been introduced and naturalized to the Mediterranean climate of southern California (Dowell and Gill, 1989). One obvious aspect of controlling non-native species introductions is stopping their entry into California. Another important consideration is the maintenance of healthy and intact native ecosystems to combat the establishment of non-natives. It has been shown

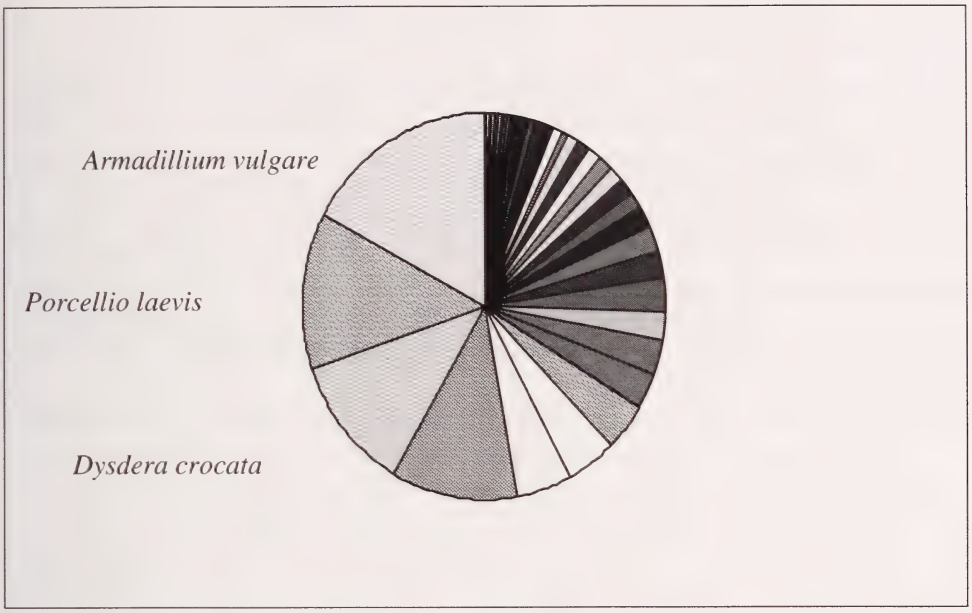

Fig. 1. Number of individuals (n=2590) per species. The three most commonly encountered species are indicated, all of which are non-native. *Armadilium vulgare* comprises 17.1%, *Porcelio laevis* 13.1%, and *Dysdera crocata* 11.4% of all species respectively.

that large and widespread stands of non-native vegetation, such as *A. donax*, displace native vegetation and allow for non-native species to dominate an area (Bell 1994). In this study, 43% of all captures by abundance were represented by four non-native species (Fig. 1). The two highest species captures in this study by abundance were Armadillium vulgare (17%), and Porcellio laevis (13%). Bolger et al. (2000) had abundances of 24.8% and 5.4% respectively for these same two taxa when sampling throughout the greater San Diego County region. Indeed, while canopies of *A. donax* and native riparian gallery trees differ greatly, the physical characteristics between habitat types are more similar on the ground. Herrera and Dudley (2004) found that captured arthropods in *A. donax* were larger and more generalized than those found in native riparian vegetation. Thus, opportunistic and generalized non-native arthropods may be more tolerant of degraded conditions and form an invasion complex (Dudley and Collins 1995).

Several new findings as a result of this study indicate the importance of continued studies on the invertebrates of southern California, and shed new light on those that are associated with dense stands of *A. donax*. These novel findings include a grub in the family Lygaeidae that was collected from Arundo and is known to be a non-native species detected in southern California only in the last 5 years (D. Faulkner, pers. comm.). Also, a grub that is commonly associated with oak and pine forests was also found in association with *A. donax*. Several members of the spider species Clubiona californica were found, and these represent a new county record for this California native.

The most significant abundance of native species found in association with *A. donax* was two species of Spirobolid millipedes that combined to make up over 11% of all captures by abundance. Millipedes as a group are generalized detritovores associated with damp or moist areas where they can feed on rotting plant material. While it has not been shown that these millipedes feed on *A. donax* , this is quite likely the case given their

abundance in an entirely *A. donax* dominated habitat type such as that sampled in this study.

The non-native Argentine ants (Linepithema humile) were the only ants collected in this study, although other species of ants, both native and nonnative, were active nearby at this time of year (unpub. data). This finding along with other studies indicates that Argentine ants establish themselves readily in non-native dominated or disturbed habitats. Bolger et al. (2000) showed that argentine ants pose a considerable threat to the arthropod fauna of southern California. Suarez et al. (1998) showed that argentine ants are more abundant in areas dominated by non-native vegetation. Since these ants were commonly found in rhizome clumps, this species may be having a deleterious impact on native species at the study site, and indirectly maintain the abundance of non-native species encountered in this study.

The impacts of dense stands of *A. donax*, and large-scale vegetation removal projects on sensitive species are poorly known. There is some evidence that *A. donax* poses a threat to the endangered least bell's vireo (*Vireo bellii pusillus*; Kisner 2004), and three-spine stickleback (*Gasterosteus* aculeatus; Frandsen and Jackson, 1994). While the three-spine stickleback is now absent from the Santa Margarita River, the least Bell's vireo and endangered arroyo toad (*Anaxyrus californicus*) are still present. Recently, removal of *A. donax* from this area has been accomplished by means of heavy equipment and the use of a grappler. While there were no arroyo toads encountered during this project, that does not mean that there will be no impacts if this eradication method is used elsewhere or at a different time. The habitat in the study area is within an expansive stand of *A. donax*, which represents atypical habitat for the arroyo toad. Such dense non-native vegetation stands are likely to be prohibitive to overland movements of the arroyo toad, and may be the reason why none were encountered. Had this work been done closer to open riparian habitat or suitable upland habitat, it is likely that toads would have been encountered. Also, it must be emphasized that this study was conducted during the fall of a relatively dry year, and different climatic conditions or seasonality may produce different results. Further research is needed to effectively determine the impacts of mechanical *A. donax* eradication on the arroyo toad during different conditions, or in different locations.

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Morphometric Comparison of Blue Catfish *Ictalurus furcatus* (Lesueur, 1840) from Northern and Southern Atlantic Drainages of México

Gorgonio Ruiz-Campos,[1] María de Lourdes Lozano-Vilano,[2] and María Elena García-Ramírez[2]

[1]*Laboratorio de Vertebrados (Colección Ictiológica), Facultad de Ciencias, Universidad Autónoma de Baja California, Apdo. Postal 233, Ensenada, Baja California, 22800, México. U.S. mailing address: PMB. No. 64, P.O. Box 189003, Coronado, CA. 92178-9003, gruiz@uabc.mx*

[2]*Laboratorio de Ictiología, Facultad de Ciencias Biológicas, Universidad Autónoma de Nuevo León, Apdo. Postal 425, San Nicolás de los Garza, 66450, Nuevo León, México*

Abstract.—A morphometric comparison was performed on specimens of blue catfish (*Ictalurus furcatus*) from northern (Lower Río Bravo) and southern (Chiapas) México in order to identify diagnostic characters that allow their discrimination. The discriminant function analysis determined three characters to be highly diagnostic to separate the two groups of specimens: the southern group [SG] has a shorter anal base (mean = 3.4 times in standard length [SL], range = 3.1 to 3.7) *vs* northern group [NG] (mean = 2.9 times in SL, range = 2.7 to 3.1), a lesser head width (mean = 6.0 times in SL, range 5.1 to 6.8) *vs* NG (mean = 5.6 times, range = 5.3 to 6.0), and a lower number of anal rays (mean = 26, range = 24 to 28) *vs* NG (mean = 31, range = 29 to 34). Additionally, 14 other characters were also different ($P < 0.01$) between both groups. All these characters support the taxonomic validation of *Ictalurus meridionalis* (Günther 1864) for the individuals of SG that are currently included in *I. furcatus* Lesueur. Studies on comparative osteology and molecular genetics of both forms are needed for the clarification of their taxonomic status.

Resumen.—Se realizó una comparación morfométrica en el bagre azul (*Ictalurus furcatus*) del norte (Bajo Río Bravo) y sur (Chiapas) de México, con el objetivo de identificar caracteres diagnósticos que permitan su discriminación. El análisis de función discriminante determinó que tres caracteres son altamente diagnósticos para separar los dos grupos geográficos. El grupo sureño [GS] tiene una base anal más corta (promedio = 3.4 veces en longitud patrón [LP], intervalo = 3.1 a 3.7) *vs* grupo norteño [GN] (promedio = 2.9 veces en LP, intervalo = 2.7 a 3.1), una cabeza más angosta (promedio = 6.0 veces en LP, intervalo 5.1 a 6.8) *vs* GN (promedio = 5.6 veces en LP, intervalo = 5.3 a 6.0), y un menor número de radios anales (promedio = 26, intervalo = 24 to 28) vs NG (promedio = 31, intervalo = 29 to 34). Además, otros 14 caracteres también fueron diferentes ($P < 0.01$) entre ambos grupos. Todos estos caracteres sustentan la validación taxonómica de *Ictalurus meridionalis* (Günther 1864) para individuos del GS que son actualmente referidos como *I. furcatus* Lesueur. Estudios sobre osteología comparada y genética molecular de ambas formas son necesarios para clarificar su estatus taxonómico.

The blue catfish *Ictalurus furcatus* (Lesueur 1840) is one of the Nearctic freshwater fish species of the Atlantic slope with a distribution extended to Neotropical localities as far

southern as the Rio Usumacinta and the Rio Belize, Belize, where it was originally called *Ameiurus meridionalis* Günther 1864 [= *Ictalurus meridionalis*] (Miller et al. 2005).

This southern form was described on the basis of its lower number of anal rays, its shorter barbels and its smaller eye than the northern form (Günther 1864; Jordan and Evermann 1896–1900; Meek 1904; Alvarez del Villar 1970). However, Lundberg (1992) considered the nominal species *I. meridionalis* from the Rio Usumacinta as conspecific with *I. furcatus*, a situation that Miller et al. (2005) stressed as an interesting theme for additional study.

Our ichthyological explorations to the "Reserva de la Biosfera de Montes Azules" in the Mexican State of Chiapas, during 1979–1985 (Lozano-Vilano and Contreras-Balderas 1987) and 2004–2006 (Lozano-Vilano et al. 2007), had already detected in the field some differences in the body proportions of the southern blue catfish specimens when compared with the northern form, mainly the basal length of the anal fin, as well as the head width and length. Additionally, the geographical distribution of blue catfish in México exhibits a notable disjunct pattern in the drainages of Veracruz, where the northern and southern populations are widely separated (cf. Miller et al. 2005).

In the present work, we compared 28 morphologic characters (27 morphometric and 1 meristic) in blue catfish from northern (Lower Río Bravo) and southern (Río Lacantún) México, in order to determine the magnitude and signification of the differences.

Methods

Thirty-four individuals of blue catfish from different sites in southern (Chiapas, B in Fig. 1: 1, Río Lacanjá; 2, Río Tzendales; 3, Arroyo Miranda; 4, Río Lacantún at Estacion Chajul; 5, Río Chajul; 6, Arroyo San Pablo; 7, El Colorado; and 8, Arroyo Manzanares) and northern México (Lower Río Bravo, A in Fig. 1: 1, Rancho Taffinder; 2, Río Alamo; 3, El Astillero; 4, Garceño; 5, La Gloria; 6, Rodríguez de Anáhuac; and 7, Presa Don Martín) were chosen for the comparative analysis (Appendix 1). The range and average length of the two groups of specimens (southern and northern) were similar (Table 1).

Twenty-two body distances based on box truss protocol (Bookstein et al. 1985, Fig. 2) and five distances of the head region and the number of anal rays were considered in the analysis (Hubbs and Lagler 1947). Each specimen was measured with a digital caliper (precision 0.01 mm) connected to a personal computer. The measurements were as follows (landmark number in parenthesis): (1-2), tip snout to mouth commissure; (1-3), tip snout to nostril; (2-3), mouth commissure to nostril; (2-4), mouth commissure to dorsal fin origin; (2-5), mouth commissure to pectoral fin origin; (3-4), nostril to dorsal fin origin; (3-5), nostril to pectoral fin origin; (4-5), dorsal fin origin to pectoral fin origin; (4-6), basal length of dorsal fin; (4-7), dorsal fin origin to pelvic fin origin; (5-6), pectoral fin origin to posterior insertion of dorsal fin; (5-7), pectoral fin origin to pelvic fin origin; (6-7), posterior insertion of dorsal fin to pelvic fin origin; (6-8), posterior insertion of dorsal fin to posterior insertion of adipose fin; (6-9), posterior insertion of dorsal fin to anal fin origin; (7-8), pelvic fin origin to posterior insertion of adipose fin; (7-9), pelvic fin origin to anal fin origin; (8-9), posterior insertion of adipose fin to anal fin origin; (8-10), posterior insertion of adipose fin to posterior insertion of anal fin; (8-11), posterior insertion of adipose fin to mid caudal base; (9-10), basal length of anal fin; and (10-11), posterior insertion of anal fin to mid caudal base. Other lineal measures and counts were: head length, head width (at level of occipital), eye diameter, internostril width, interorbital width, and the number of anal rays.

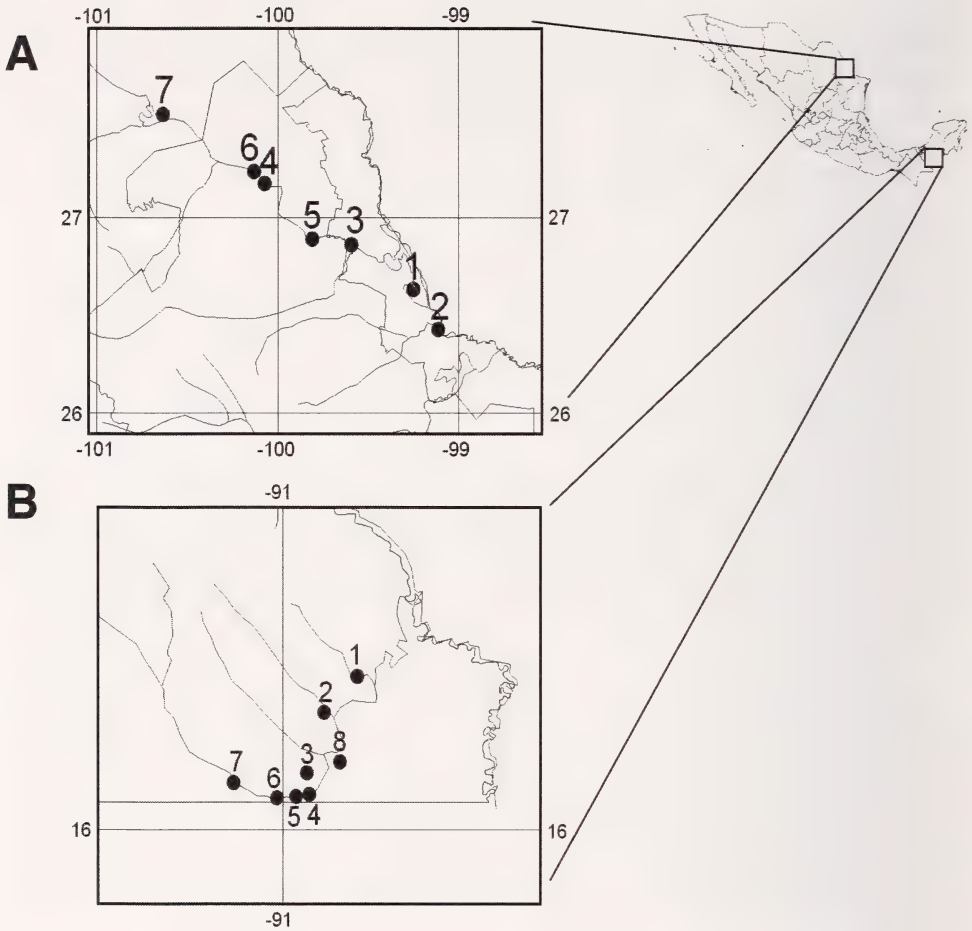

Fig. 1. Study sites for blue catfish in northern and southern México. (A) Lower Río Bravo of Coahuila, Nuevo León and Tamaulipas; (B) Río Lacantún at Reserva de la Biosfera de Montes Azules, Chiapas. Names of sites are indicated in the text.

The original body measurements were standardized by means of the regression of Elliott et al. (1995), which removes the size component from the shape measurements (allometry), and is calculated for each character by the following equation:

$Ms = Mo\ (Ls/Lt)^b$; where Ms = standardized measurement of the character, Mo = original measurement of the character (mm), Ls = average standard length (mm) of all the specimens from the two groups examined, Lt = standard length (mm) of specimen, and "b" was estimated for each character from the observed data using the non-linear regression equation, $M = aL^b$. Parameter "b" was estimated as the slope of the regression log Mo on log Lt using all fish.

The standardized morphometric values of the 34 examined specimens were analyzed between groups (northern: Lower Río Bravo basin, and southern: Río Lacantún basin) by means of "forward stepwise discriminant" function analysis (DFA) using Statistica 5.0 (StatSoft, Inc., Tulsa, OK, 1995). The DFA allowed us to detect which combination of characters discriminated best between groups. Finally, each character was compared statistically between groups using a Student's "t" test.

Table 1. Descriptive statistical values of 27 standardized morphological characters plus one meristic (anal rays), with their respective levels of significance (P), for individuals of blue catfish from northern and southern, México. Significant characters are shown in bold.

	Río Lacantún N=21		Lower Río Bravo N=13			
Variables	Ave.	SD	Ave.	SD	Student's "t"	P
SL (mm)	211	43.3	219	44.8	0.517	0.609
1-2	15.21	0.92	15.28	1.59	0.163	0.871
1-3	13.34	1.09	12.35	1.07	2.591	**0.014**
2-3	9.46	1.15	9.36	1.39	0.228	0.821
2-4	68.25	6.89	76.83	7.64	3.386	**0.002**
2-5	35.62	2.88	34.57	1.64	1.196	0.241
3-4	66.29	5.46	68.75	3.00	1.486	0.147
3-5	38.24	5.55	38.54	4.78	0.257	0.797
4-5	47.27	11.07	53.09	10.48	1.52	0.138
4-6	17.02	1.15	15.77	1.00	3.231	**0.003**
4-7	54.63	4.26	56.63	5.08	1.236	0.225
5-6	55.37	3.68	57.60	3.04	1.83	0.077
5-7	57.48	5.28	52.19	3.45	3.204	**0.003**
6-7	44.63	4.50	47.44	3.26	1.952	0.060
6-8	85.53	6.77	89.57	5.31	1.828	0.077
6-9	57.52	3.05	53.45	2.04	4.247	**<0.001**
7-8	82.47	5.07	95.17	4.19	7.562	**<0.001**
7-9	28.71	2.82	24.26	1.70	5.125	**<0.001**
8-9	60.50	3.99	77.45	6.32	9.62	**<0.001**
8-10	25.67	1.59	21.98	1.21	7.166	**<0.001**
8-11	45.94	1.94	42.90	1.69	4.656	**<0.001**
9-10	60.74	3.64	78.64	4.27	13.045	**<0.001**
10-11	34.60	3.02	27.35	2.11	7.567	**<0.001**
Head width	34.11	3.27	42.67	1.69	8.711	**<0.001**
Internostril width	16.24	0.75	14.67	0.65	6.23	**<0.001**
Head length	49.99	2.18	54.08	3.11	4.512	**<0.001**
Interorbital width	26.15	1.10	28.05	0.92	5.196	**<0.001**
Eye diameter	9.13	0.56	9.23	0.81	0.426	0.673
Anal rays	26	1	31	2	9.719	**<0.001**

SL (mm) = no transformed standard length.

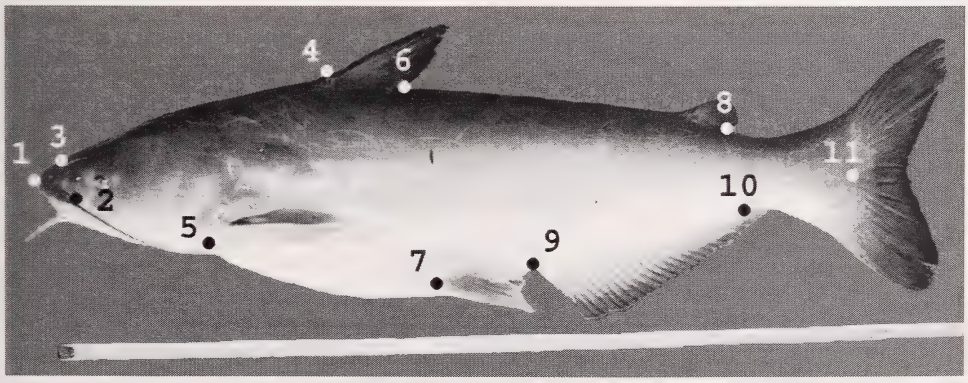

Fig. 2. Landmarks based on Bookstein et al. (1985) box truss protocol for the morphometric comparison of groups of blue catfish from northern and southern México. Photograph by María de Lourdes Lozano-Vilano.

Table 2. Summary of the discriminate function analysis (forward stepwise type) applied for standardized measurements of blue catfish from northern (Lower Río Bravo basin) and southern (Río Lacantún basin). Number of steps: 9; number of variables in the model: 9; grouping: 2.

Character	Wilks	Partial	F-remove	p-level	Toler.	1-Toler.
9-10	0.036161	0.831570	4.86106	0.037299	0.522359	0.477641
8-10	0.057616	0.521912	21.98478	0.000092	0.352112	0.647888
Head width	0.043603	0.689646	10.80049	0.003113	0.363757	0.636243
6-7	0.032274	0.931708	1.75914	0.197216	0.552287	0.447713
Internostril width	0.032742	0.918411	2.13209	0.157211	0.709333	0.290667
Interorbital width	0.033956	0.885572	3.10113	0.090976	0.764098	0.235902
Anal rays	0.032556	0.923661	1.98355	0.171840	0.882346	0.117654
2-5	0.032599	0.922422	2.01848	0.168258	0.458300	0.541700
7-9	0.031405	0.957506	1.06513	0.312338	0.706935	0.293065

Overall Wilks' Lambda: 0.03007. $F_{approx.\ (9,\ 24)} = 86.014$, $p < 0.0001$.

Standardized coefficients for canonical variables

Variable	Root 1
9-10	0.57657
8-10	−1.18316
Head width	0.93789
6-7	−0.35705
Internostril width	−0.34437
Interorbital width	0.39294
Anal rays	0.29866
2-5	0.41776
Distance 7-9	−0.24895
Eigenval	32.25540
Cum. Prop.	1.00000

Results and Discussion

The means and standard deviations of the 28 morphological characters of 34 examined specimens of blue catfish are depicted in Table 1. The "t" student test for each character between groups (northern *vs.* southern) revealed 17 characters to be statistically different.

The DFA applied to 34 specimens of blue catfish from both groups selected 9 of 28 morphologic variables examined (Table 2). Overall value of Wilks' lambda was 0.03007, indicating a significant discrimination ($p<0001$) between groups. The highest Wilks' values were associated with the basal length of anal fin (0.0361), posterior insertion of adipose fin to posterior insertion of anal fin (0.0576), and the head width at level of occipital (0.0436). The contribution of each one of the nine characters selected by the model to the overall discrimination appears in the Table 2. The characters with high weight to the discrimination between groups were the variables: 8-10 ($Y_1 = -1.18316$), head width ($Y_1 = 0.93789$) and 9-10 ($Y_1 = 0.57657$). Predicted or correct classification of individuals was 100% in both groups, which indicates that the individuals maintain the identity of each group as shown in figure 3.

The count of anal rays was statistically different between groups, with the lowest number (mean = 26, range = 24 to 28) for the southern group (SG) and the highest for the northern group (NG, mean = 31, range = 29 to 34) (Table 1). When both groups

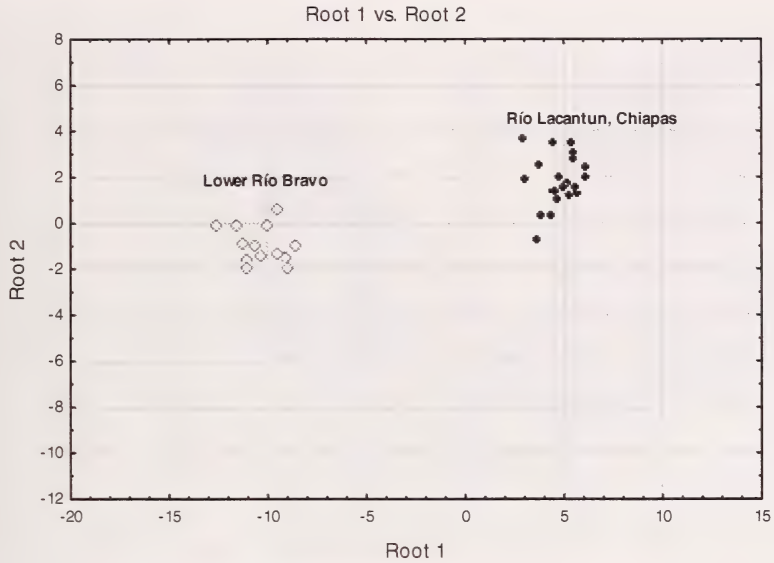

Fig. 3. Discriminant function analysis for northern and southern blue catfish specimens from México. Axis 1 vs. axis 2. Pooled data.

were compared without transformation of the body measurements, the following body proportions were obtained: in the NG, the head width contained in average 5.6 times in standard length [SL] (range 5.3 to 6.0), while in that of the SG 6.0 times (range 5.1 to 6.8). The head of the NG specimens was notably longer and wider than in those SG specimens (Fig. 4). The basal length of anal fin in the NG specimens contained in average 2.9 times (range = 2.7 to 3.1) in SL in comparison with that of the SG (3.4 times, range = 3.1 to 3.7).

In the comparative analysis of blue catfish between specimens from the northern and southern populations of México (Lower Río Bravo and Río Lacantún, respectively) 17 morphological characters were found to be significantly different (Table 1). Several of these differences had already been referred in the literature (e.g., Günther 1864; Jordan and Evermann 1896–1900; Meek 1904; Alvarez del Villar 1970), such as a lower number of anal rays and a smaller head in southern populations (*I. "meridionalis"*) in comparison with those of northern populations (*I. furcatus*). Also, the length of the barbels is different between these two populations; being longer in northern specimens (reaching the origin dorsal fin) and shorter in southern specimens (reaching the end of head or slightly beyond).

We also detected that the coloration of live specimens of "*I. meridionalis*" is gray silvery on the dorsal part of the body with steel reflections and whiter ventrally; however, this coloration pattern is different than that reported by Jordan & Evermann (1896) and Meek (1904), which was brownish with steel blue reflections on the dorsum, and silvery on the ventral region. Of the 17 characters found to be significant by means of the student's "t" test, three of them were highly useful for separating both groups and were associated with distances in the anal region and the head width. Although the number of anal rays could be a character with latitudinal clinal variation, we did not detect this trend because specimens examined here from an intermediate area (Rio Tanquilin, San

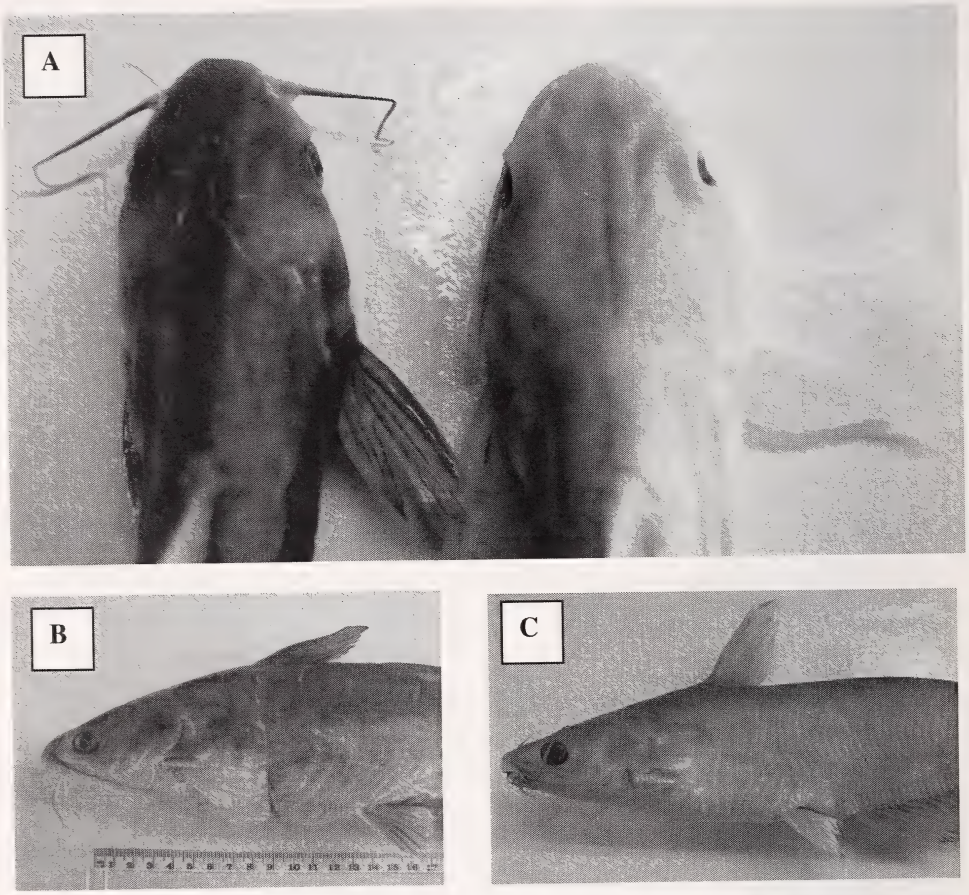

Fig. 4. (A) Dorsal cephalic views of blue catfish for southern specimen (left) and northern (right). Lateral cephalic views of southern specimen (B) and northern specimen (C). Photographs by Gorgonio Ruiz-Campos.

Luis Potosí) had a lower number of anal rays (mean = 22) when compared with southern specimens of Chiapas (mean = 26).

In spite of the range and average of length for both species examined being statistically similar, we expect that the variation of each character compared will decrease as the sample size increases. We suggest that a larger sample size for each southern and northern group, including intermediate populations, as well as a detailed study on the osteology and molecular genetic analysis should be conducted to evaluate the taxonomic status of the southern form "*Ictalurus meridionalis*."

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Appendix 1

The material examined of blue catfish for the comparative analysis is deposited in the Colección Ictiológica del Laboratorio de Ictiología, Facultad de Ciencias Biológicas, Universidad Autónoma de Nuevo León (UANL). CHIAPAS: UANL-15739, Río Tzendales, 16°17′52″ N, 90°53′12″ W, 24 Aug. 2004; UANL-15718, Arroyo San Pablo, 16°06′07″ N, 91°00′52″ W, 23 Aug. 2004; UANL-15790, *idem*, 30 Nov. 2004; UANL-16984, Arroyo San Pablo, *idem*, 10 Dic. 2005, 30 Nov. 2004; UANL-16883, Río Manzanares, 16°10′14″ N, 90°50′36″ W, 14 Sep. 2005; UANL-15760, Arroyo Miranda exit to Lacantún, 16°08′44″ N, 90°55′50″ W, 25 Aug. 2004; UANL-15822, Río Lacantún at Estación Chajul, 16°06′35″ N, 90°56′23″ W, 1–2 Dec. 2004; UANL-15993, Río Lacanjá, 16°24′14″ N, 90°47′52″W, 10 Feb. 2005; UANL-16698, Río Lacanjá, *idem*, 9 Jun. 2005; UANL-16726 (ex UANL 16716), El Colorado, 16°07′13″ N, 91°07′50″ W, 9 Jun. 2005; UANL-16883, Río Manzanares, 16°10′14″ N, 90°50′36″ W, 14 Sep. 2005; UANL-17046, Río Chajul, 16°06′11″ N, 90°57′22″ W, 11 Dec. 2005. TAMAULIPAS: UANL-826, Rancho Taffinder, 8 km NNW Nueva Ciudad Guerrero, 26°37′42″N, 99°14′59″W, 14 Oct. 1966; UANL-4226, mouth of the Río Alamo, 5.2 km E Ciudad Mier, 26°25′34″N, 99°06′41″W, 15 Feb. 1982; UANL-8968, Río Salado at El Astillero, 26°51′30″N, 99°35′28″W, 6 Jun. 1985; NUEVO LEON: UANL-8157, Río Salado at Garceño, 27°10′19″N, 100°04′10″ W, 7 Ago. 1984; UANL-8557, Río Salado at La Gloria,

26°53′14″N, 99°48′20″W, 10 Nov. 1984; UANL-8935, Río Salado at La Gloria, *idem*, 6 Jun. 1985; UANL-11628, Río Salado at Rodríguez de Anáhuac, 27°13′59″N, 100°07′43″W, 1982 [no date]; COAHUILA: UANL-15179, Presa Venustiano Carranza (Don Martín), 27°31′24″N, 100°37′51″W, 9 May 2002. Other material examined. VERACRUZ: UANL-1823, Río Papaloapam at Los Amates, 18°17′00″ N, 95°52′00″ W, 14 Dec.1972. TABASCO: UANL-2911, Río Sonapa 18 Km W Huimanguillo, 17°52′00″N 93°28′00″W ,14 Feb. 1978. SAN LUIS POTOSI: UANL-1269, Río Tanquilin SW Camoca, 21°16′00″ N, 99° 03′ 00″ W, 28 Oct., 1971.

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McWilliams, K. L. 1970. Insect mimicry. Academic Press, vii+326 pp.

Holmes, T. Jr., and S. Speak.1971.Reproductive biology of Myotis lucifugus. J. Mamm., 54:452–458.

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Cover: Blue catfish (*Ictalurus furcatus*). Photo by María de Lourdes Lozano-Vilano.